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A STUDY OF THE PATHOGENICITY OF THE MEADOW NEMATODE AND ASSOCIATED FUNGUS *CYLINDROCARPON RADICICOLA* WR.[1]

BY R. J. HASTINGS[2] AND J. E. BOSHER[2]

Abstract

The meadow nematode *Pratylenchus pratensis* has been isolated from root lesions of narcissus, strawberry, apple, cherry, and raspberry in British Columbia. When apparently freed from associated fungi, this species reduced the growth of potato, carrot, red clover, tomato, spinach, and violet seedlings by 50 to 75%, and oat seedlings by less than 4%. In parallel experiments, the commonly associated fungus *Cylindrocarpon radicicola* as a pure culture reduced growth by only 6 to 11%. The inhibition of growth by the fungus and nematode as a mixed culture was usually greater than the sum of the reductions as pure cultures.

The nematodes were separated from associated fungi by planting segments of infested oat roots in plate cultures of powdered peat moistened with a 0.1% solution of malachite green. When the oats planted in this medium had germinated, the nematodes moved from the root segments through the fungicidal medium into the roots of the oat seedlings. These nematode-infested roots proved to be free from the fungi and bacteria naturally associated with the nematode in field infestations.

The adult and all the larval stages of *P. pratensis* proved to be capable of entering the roots of oat seedlings.

Introduction

The occurrence of the meadow nematode (*Pratylenchus pratensis* (de Man 1880) Filipjev 1936) as a root parasite in British Columbia and elsewhere has been reported (1-7), but the pathogenicity of the nematode has never been satisfactorily established. Ark and Thomas (1) compared the growth of apple seedlings in healthy soil and soil inoculated with *P. pratensis*. They found that the nematodes caused considerable stunting, but pointed out that the degree of injury could not be determined with certainty owing to the lack of effectual methods for freeing the nematodes from associated bacteria and other organisms.

No investigator has presented convincing evidence that *P. pratensis* is definitely pathogenic when freed from associated organisms. This species is an obligate parasite which is found in the roots of a wide range of plants and in the soil. When found within plant roots it is nearly always associated with other organisms, therefore infested roots cannot be considered as a satisfactory source of inoculum owing to the presence of these associates.

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Contribution No. 546 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

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Experimental

Hosts and Host Preferences of the Meadow Nematode

Attempts were made to develop *P. pratensis* in pure culture upon artificial media without success, hence roots had to be used as a source of inoculum. The preference of the nematodes for the roots of specific plants was investigated to establish the relative value of the roots as living media in the multiplication of the nematodes, and at the same time to obtain information on the relative susceptibility of different species attacked by the meadow nematode. The average numbers of nematodes that entered the roots of different species are given in Table I.

TABLE I

THE AVERAGE NUMBER OF MEADOW NEMATODES THAT ENTERED SEEDLINGS GROWN IN INFESTED STRAWBERRY SOIL

Host	Age of seedlings, days	Number of nematodes in each seedling
Common vetch	8	52
Red Fife wheat	8	3
Early blue pea	8	53
Banner oats	8	208
Barks barley	8	8
Sunflower	8	25
Japanese millet	8	5
Yellow eye bean	9	37
1000-headed kale	9	86
Red clover	9	14
Earliana tomato	16	12
Carrots	16	12
Timothy grass	24	26

The data revealed that all species tested were susceptible. The large numbers found in oats suggested that *P. pratensis* is especially attracted to the roots of this seedling under the growth conditions of our greenhouse.

The affinity of oat seedlings for the nematode can be used to reveal the field distribution, by planting oats on collected soil samples. The number of nematodes in oat seedlings grown on soil samples from an infested strawberry plantation showed that *P. pratensis* was more abundant under the strawberry plants than midway between the rows.

Fungicidal Aids in Freeing the Meadow Nematode from Associated Organisms

Malachite green was selected as a fungicide to free oat roots infested with *P. pratensis* from associated organisms, because of the high tolerance of both oats and the nematode to this chemical. Newton and Edwards (8) have reported that 0.11% is lethal to yeast and *Bacterium Juglandis*. Munro and Newton (9) found that 0.001% malachite green inhibited the growth of *Fusarium culmorum*, *F. moniliforme*, *F. Solani*, *F. orthoceras*, *Pythium ultimum*, and *Rhizoctonia Solani*. Verona (10) found that a concentration of 0.001% arrested the germination of spores of *Tilletia levis*, *T. caries*, *Ustilago Maydis*, and *U. Tritici*, while Verona and Ceccarelli (11) established that 0.0005% inhibited the growth of *Verticillium Amaranti*, *V. albo-atrum*, *V. Dahliae*, and *V. tracheiphilum*.

Nematode-infested oat root segments were placed in a medium containing 0.4 gm. each of disodium phosphate, magnesium sulphate and potassium nitrate, 40 gm. of sucrose, 20 gm. of agar, and varying amounts of malachite green per litre of distilled water. The survival of bacteria, fungi, and nematodes was studied in these cultures. The results are given in Table II.

TABLE II

THE GERMICIDAL AND FUNGICIDAL EFFICIENCY OF MALACHITE GREEN SHOWN BY THE SURVIVAL OF ORGANISMS IN OAT ROOT INOCULATIONS

Conc. of malachite green, %	Organisms that developed in cultures			Remarks
	Bacteria	Fungi	Nematodes	
0.001	+++	++	++	*Fusarium* sp.; *Cylindrocarpon* sp. and bacteria were found.
0.005	+++	++	+	*Fusarium* sp.; *Cylindrocarpon* sp. and bacteria were found.
0.010	++	±	+	Bacteria only were abundant.
0.050	±	−	+	
0.100	−	−	+	
0.500	−	−	+	

The minimum inhibitory concentrations of malachite green were 0.05% for fungi and 0.1% for bacteria. These values are somewhat higher than those reported by Munro and Newton (9) and Verona and Ceccarelli (10, 11), possibly owing to dye absorption by the root tissue.

Tolerance of Meadow Nematode and Oat Seedlings to Malachite Green

When pieces of nematode-infested roots were placed in the middle of a Petri dish, in a medium of peat saturated with 0.1 to 0.6% malachite green and planted with oats, the nematodes moved from the root inoculum through the fungicidal medium into the roots of the oats when the seeds germinated. These peat cultures were used to establish the tolerance of the seedlings and the nematodes to malachite green. The results are shown in Table III.

TABLE III

THE TOLERANCE OF OATS AND THE MEADOW NEMATODE TO MALACHITE GREEN AS SHOWN BY SEEDLING GROWTH AND THE NUMBER OF NEMATODES THEREIN

Conc. of malachite green, %	Seedling growth			Average number of nematodes in a seedling
	Germination, %	Length of top, cm.	Length of root, cm.	
0.01	100	11	3.0 – 4.5	56
0.05	90	11	3.0 – 4.5	53
0.10	90	11	3.0 – 4.5	53
0.20	90	11	2.5 – 4.0	21
0.40	100	8.5	2.0 – 3.0	9
0.60	70	7.0	0.5 – 1.0	5
0.80	100	6.0	0	0

Oats were tolerant of a concentration of malachite green up to 0.1%, but above this concentration the growth was retarded. When root growth occurred, the nematodes moved from the excised root segments to the roots of the living seedlings. Peat cultures containing 0.1% malachite green proved to be the most satisfactory medium for obtaining nematode-infested oat seedlings free from the organisms usually associated with the meadow nematode.

Pathogenicity Studies

The pathogenicity of the organisms towards various species of plants was measured by the growth reduction of seedlings in inoculated soil compared with those raised on steamed soil.

TABLE IV

THE EFFECT OF INOCULATING SOIL WITH *P. pratensis* AND *C. radicicola* ON THE GROWTH OF VARIOUS SEEDLINGS

Crop	Inoculum	Age of plants, days	No. of plants alive	Average weight, gm.	Relative growth, %	Reduction in growth, %
Irish potato	Check	72	17	0.3127	100	—
	C. radicicola	72	20	0.2788	89.1	10.9
	P. pratensis	72	15	0.1266	40.4	59.6
	C. radicicola and *P. pratensis*	72	13	0.0557	17.8	82.2
Carrot	Check	53	15	0.2170	100	—
	C. radicicola	53	15	0.1926	88.7	11.3
	P. pratensis	53	17	0.0923	42.5	57.3
	C. radicicola and *P. pratensis*	53	13	0.5500	25.3	74.7
Red clover	Check	37	10	0.4530	100	—
	C. radicicola	37	16	0.4771	105.3	—
	P. pratensis	37	11	0.1510	35.1	64.9
	C. radicicola and *P. pratensis*	37	16	0.0706	16.4	83.6
Tomato	Check	30	12	0.6137	100	—
	C. radicicola	30	12	0.5759	92.9	7.1
	P. pratensis	30	6	0.1642	26.7	73.3
	C. radicicola and *P. pratensis*	30	11	0.1818	29.6	70.4
Spinach	Check	10	10	0.2920	100	—
	C. radicicola	10	11	0.2730	93.5	6.5
	P. pratensis	10	7	0.0960	32.8	67.2
	C. radicicola and *P. pratensis*	10	7	0.0860	29.5	70.5
Violet	Check	87	4	1.0875	100	—
	C. radicicola	87	4	1.1142	102.4	—
	P. pratensis	87	4	0.2550	23.4	76.6
	C. radicicola and *P. pratensis*	87	4	0.2125	19.5	81.5
Oats	Check	73	10	2.0730	100	—
	C. radicicola	73	10	1.9090	92.0	8.0
	P. pratensis	73	10	1.9970	96.3	3.7
	C. radicicola and *P. pratensis*	73	10	1.7340	83.7	16.3

P. pratensis cultures, purified by passage through peat to oat seedlings in the presence of 0.1% malachite green, were used as the nematode inoculum. Ten infested seedlings were mixed with the soil in a 4-in. pot.

A culture of *Cylindrocarpon radicicola* was grown on steamed whole barley, and four grains served as the fungus inoculum for each 4-in. pot.

The results of these studies are reported in Table IV.

The experiments showed that *P. pratensis* reduced the growth of potato, carrot, red clover, tomato, spinach, and violet seedlings by 50 to 75%, but oat seedlings by less than 4%. No satisfactory explanation can be offered as to why the pathogenicity of the nematode to oats is not more pronounced. Shortly after germination, more nematodes are found in oat roots than in other common crops, as will be seen by referring to Table I. A possible explanation is that the total nematode population of the soil enters the seminal roots, thus allowing the nodal roots to develop.

C. radicicola reduced the growth of the seedlings by 6 to 11% only.

The two organisms as a mixed culture usually caused more damage than the sum of the damage caused by pure cultures.

The Infective Stage of the Meadow Nematode

The life history of *P. pratensis* is not completely known, and Goodey (12, pp. 111-116) surmised that the first stage larva is the probable infective stage. Our experiments reveal that all stages are capable of entering oat roots. Oat seeds sown in a malachite-green peat medium germinated between the third and fourth days. On the fourth day, when the day-old roots were only 2 cm. long, they were found to contain adult as well as larval specimens of *P. pratensis*. Eggs were found in 3-day-old roots. The ability of adult and other stages to invade roots means that large nematode populations in root tissue may be built up rapidly by mass infection from the soil, and by immediate multiplication within the plant.

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THE NATURE OF BULB NEMATODE (*DITYLENCHUS DIPSACI*) POPULATIONS IN "SUPREME", "PRINCE ALBERT", AND "IMPERATOR" IRIS BULBS, AND THEIR CONTROL BY THERMAL TREATMENT[1]

By R. J. Hastings[2] and J. E. Bosher[2]

Abstract

"Supreme" and "Prince Albert", representing a Dutch *tingitana* hybrid and an English iris, are much more susceptible to nematode infestation than "Imperator", a Dutch iris, as judged by the number of nematodes per unit volume of invaded tissue, *viz.*: 596, 108, and 13 respectively. Infestation in "Prince Albert" tends to be confined to the basal plate. Rapid multiplication of the nematodes within the bulb tissue occurs in "Supreme" and "Prince Albert." On the other hand, little multiplication occurs within "Imperator" bulb tissue.

The populations within iris bulb tissue consist largely of young larvae in contrast with a high pre-adult population in narcissi. The low population of the heat-resistant pre-adults accounts for the fact that the nematode population in "Supreme" bulbs can be destroyed by a 60-minute immersion at 110° F., whereas a three-hour immersion is required to destroy the nematodes in narcissi.

Introduction

The symptoms caused by the bulb nematode (*Ditylenchus dipsaci* (Kühn) Filipjev 1936) in iris bulbs differ with species and variety. Steiner and Buhrer (1) reported nematode infestations in more than 22 varieties of irises including Dutch *(I. xiphium hybridum)*, English *(I. xiphioides)*, Spanish *(I. xiphium)* and Tangerian *(I. tingitana)* irises. They considered that the symptoms were the same or very similar in all four species, and that five to six months is sufficient time for an infestation to kill a bulb.

Our observations reveal that bulbs of the *tingitana* hybrid "Supreme" are often killed in a few months, while under the same conditions those of the Dutch iris "Imperator" are little damaged. Bulbs of the English iris "Prince Albert" may have their basal connective tissue completely invaded and destroyed without any evidence of attack on the bulb scales.

Experimental

A study of the progress of nematode infestation in iris bulbs and of the nematode populations in invaded tissue indicates that the nematodes first enter and become established in the basal plate of "Supreme". Rapid multiplication occurs in the basal plate tissue; from this region they penetrate into the bulb scales, and continue to advance and multiply until the whole bulb becomes spongy in texture, gray in color, and shrivelled in appearance. Among infested lots of "Supreme" bulbs, mummified specimens and others with large gray areas are commonly found at planting time.

The nematodes also enter the basal plate of "Imperator" and eventually work their way into the bulb scales, but little multiplication occurs in either

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basal tissue or bulb scale. Hence the nematode populations are generally small and cause only a few streak lesions.

The nematodes entering the basal plate of "Prince Albert" breed and deposit many eggs. Under storage conditions the basal tissue becomes too dry for normal development, hence the populations in this variety rarely reach the size found in "Supreme". The basal tissue of "Prince Albert" is very large and there is seldom time for the infestation to progress beyond the basal plate. The damage sustained is usually confined to the basal tissue.

Since the mother bulb of the iris disappears when the new bulbs develop, a nematode infestation is the result of infestation during the current season. The consistently large nematode populations of "Supreme" and "Prince Albert" and small populations of "Imperator" are probably due to the relative ability of the respective tissue to support a nematode population. Kreis (3) studied the populations of *D. dipsaci* infesting sweet potatoes, and reported that the population in the outer 1.5 mm. of the tuber was more than 15 times greater than that of the inner layers. He suggested that the distribution of richer and more suitable food material near the skin is the probable reason. The appearance of a nematode infestation in the three classes of bulbs investigated by the writers is shown in Fig. 1.

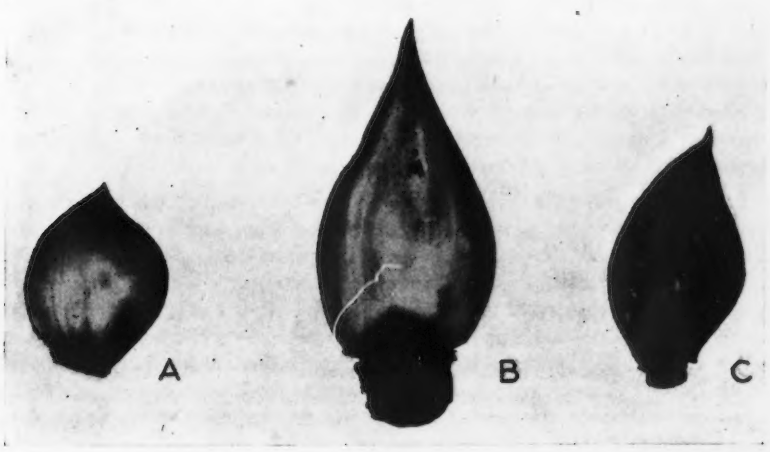

FIG. 1. *The injury caused by D. dipsaci to iris bulbs. A. Small streak lesions in fleshy scale of Dutch iris var. "Imperator". B. Sponginess and discoloration of basal tissue of English iris var. "Prince Albert". C. Sponginess and discoloration of entire outer fleshy scale of tingitana hybrid, var. "Supreme".*

A quantitative study of the nematode populations in the three classes of bulbs was made by suspending approximately 5 cu. mm. of macerated bulb tissue from each in 20 cc. of tap water, followed by a count of the nematodes in 10 microscopic fields of approximately 2.6 sq. mm. area. Although

"Supreme", "Prince Albert", and "Imperator" represent three bulb classes, the analysis of their nematode populations as given in Table I may represent varietal rather than class distinctions in susceptibility.

TABLE I

COUNTS OF NEMATODES IN SUSPENSIONS OF IRIS TISSUE

"Imperator"				"Supreme"				"Prince Albert"			
Adult	Pre-adult	Larvae	Eggs	Adult	Pre-adult	Larvae	Eggs	Adult	Pre-adult	Larvae	Eggs
1	0	0	0	0	1	68	3	2	0	17	18
0	1	1	0	0	0	40	2	2	2	11	31
0	0	1	0	2	1	65	1	3	0	15	45
0	0	1	0	0	0	53	2	1	1	4	16
0	1	2	0	0	0	58	3	2	1	5	34
1	0	1	0	1	1	48	0	2	4	3	19
0	0	0	0	1	0	59	4	2	0	4	45
0	0	0	0	1	1	69	2	1	2	5	45
0	1	0	0	1	0	66	1	1	3	5	46
1	0	1	0	2	0	48	1	3	3	4	42
3	3	7	0	8	4	584	19	19	16	73	341

The resistance of "Imperator" tissue to the bulb nematode is suggested by the small numbers in the lesions, a total of 13 in contrast with 596 in "Supreme", and 108 in "Prince Albert". The susceptibility of the last two is further emphasized by the fact that the invaded zone was many times greater than in the first-named. Infested "Supreme" and "Prince Albert" bulbs are a much greater menace as sources of inoculum than "Imperator", for they usually carry greater nematode populations.

In contrast with the large number of pre-adult nematodes in narcissus bulbs, the populations in iris consist largely of eggs and immature larvae. Since the pre-adult larvae are more resistant to heat than the eggs and immature larvae (2) it follows that iris need not be subjected to the prolonged heat treatment prescribed for narcissi. While our investigations show that immersion for three hours at 110–112° F., preceded by a 12-hour immersion in water at room temperature, is necessary for narcissi, a one-hour immersion at the same temperature effectively destroys the nematodes in iris bulbs when the treatment is preceded by a 2.5-hour immersion in water at room temperature.

"Supreme" bulbs were immersed in water at 110° F. for varying periods, and the treated tissue was macerated and suspended in water for microscopic examination.

More than 95% of the nematodes in the control were motile; but less than 0.1% were motile after the bulbs were immersed in hot water for 45 min., and none survived 60 min. A one-hour immersion of infested iris bulbs in water at 110° F. is apparently sufficient to destroy the nematode popula-

tions in iris, even when the bulbs are not pre-soaked to increase the susceptibility of the populations to heat.

TABLE II

THE MOTILITY OF BULB NEMATODES IN INFESTED "SUPREME" BULBS AFTER TREATMENT IN WATER AT 110° F. FOR VARYING PERIODS

Class of nematodes	Time of immersion in minutes								
	0	30	45	60	75	90	105	120	150
Adults	++++	+	±	−	−	−	−	−	−
Pre-adults	++++	+	±	−	−	−	−	−	−
Larvae	++++	+	−	−	−	−	−	−	−

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OBSERVATIONS ON THE STUDY OF VARIETAL DIFFERENCES IN THE MALTING QUALITY OF BARLEY. PART II[1]

By J. Ansel Anderson[2] and Henry R. Sallans[2]

Abstract

Samples of O.A.C. 21 and Wisconsin 38 barley from two stations were germinated at 56° and 50° F. with 44.5% moisture, and with 44.5 and 42.5% moisture at 53° F. Aliquots were kilned and analyzed after 3, 5, 7, 9, 11 and 13 days. Data for extract, diastatic power, and permanently soluble nitrogen, as percentage of wort solids, were plotted against time. Both varieties responded in almost exactly the same manner to changes in temperature and moisture. Values for O.A.C. 21 were consistently higher, but paired curves, representing samples of both varieties from the same station, became closer with increasing time, owing largely to overmodification of the O.A.C. 21. A real difference in malting quality between these two varieties, greater than the differential effect of malting method on them, is therefore indicated.

In the first paper of this series (3) it was pointed out that three main factors affect the determination of varietal differences in the malting quality of barley. These factors are: (i) the precision of the malting test; (ii) the differential effect of environment on varieties and (iii) the differential effect of malting method on varieties.

Investigation (2, 3, 5) has convinced us that the first of these is of minor importance. A considerable body of data on the effect of the second factor is rapidly being accumulated in several countries (3, 4, 6–9, 11, 12). This shows that the differential effect of environment on varieties is definitely a major complicating factor in studies of malting quality. In consequence, broad generalizations are justified only with respect to differences between certain varieties, the relative performance of others being considerably affected by the prevailing environmental conditions.

The third factor, the differential effect of malting method on varieties, has received little direct study although information concerning it is available as a side-product of two recent investigations. Berglund (6) had samples of several varieties malted in three commercial plants. It seems safe to assume that all plants used similar methods, designed for malting the two-rowed barleys under test to good advantage, and thus no very considerable differential effect of method on varieties would be expected. The data show that the effect was very small. A similar limitation existed in our own study (3) in which laboratory and stocking methods, which closely simulated commercial practice, were used. In these circumstances, it is not surprising that the available data suggest that the differential effect of malting method on varieties is by no means as large as the differential effect of environment.

[1] *Manuscript received May 12, 1938.*
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[2] *Biochemist, National Research Laboratories, Ottawa.*

It is difficult to elucidate the effects of malting methods on different varieties when comparisons are confined to analyses of samples of the finished malt. It appears, however, that the difficulty can be largely overcome by withdrawing and analyzing aliquots of the samples at intervals during the germination period. The effects of malting methods can then be represented by curves rather than by single points and interpretation of results is facilitated. Thunaeus and Schröderheim (12) have used this technique for comparing different varieties under the same malting conditions, while Piratzky and Rehberg (10) and earlier investigators have used it in studying the effects of different malting conditions on samples of one or more commercial barleys.

In laboratory malting studies, the capacity of the equipment sets certain limits to the number of samples which can be studied to advantage by this method. Moreover, its use creates a relatively large increase in the work required for the study of each sample. For these reasons the present investigation was limited to samples of two varieties of barley from two stations.

One of these varieties, O.A.C. 21, represents the Manchurian class of closely related six-rowed, rough-awned varieties, which has been found satisfactory for malting purposes in Canada. The other, Wisconsin Pedigree 38, represents a comparatively new class of smooth-awned, six-rowed varieties, having Lion as one of the original parents. Routine laboratory malting tests (4, 8, and unpublished results) have shown that these smooth-awned barleys are characterized by low extract yield and enzymatic activity. Canadian maltsters do not like them.

In the investigation reported in this paper, an attempt was made to determine whether the differences between O.A.C. 21 and Wisconsin 38, which are demonstrated under standard conditions in the routine test, would persist when the varieties were compared over a range of malting conditions. Although definite conclusions can hardly be drawn from the study of only two samples of each, the data appear to be of sufficient interest to merit publication. Moreover, they provide information that can be used to advantage in interpreting the results of further studies in this series, in which only two germination times are used.

Materials and Methods

Comparable samples of O.A.C. 21 and Wisconsin Pedigree 38 barley were obtained from Indian Head, Saskatchewan, and from Nappan, Nova Scotia. The samples had the following nitrogen contents:— O.A.C. 21, from Indian Head, 2.51%, and from Nappan, 1.39%; Wisconsin 38, from Indian Head, 2.77%, and from Nappan, 1.43%.

Portions representing 1 kg. of dry matter, distributed equally among four cages, were malted in equipment at the National Research Laboratories (1) under the following germination conditions:— 56° F. (chamber temperature) and 44.5% moisture (in steeped barley), 50° F. and 44.5% moisture; 44.5% moisture and 53° F., and 42.5% moisture and 53° F. Aliquots representing one-sixth of the original were removed and kilned under uniform conditions after 3, 5, 7, 9, 11 and 13 days in the germination chamber. The samples of

barley grew continuously and steadily during the whole 13 days, and it was not necessary to sprinkle any of them. Duplicate maltings were not made since it appeared that the smoothness of the curves would provide an adequate estimate of precision. Analyses were made by methods previously described (2).

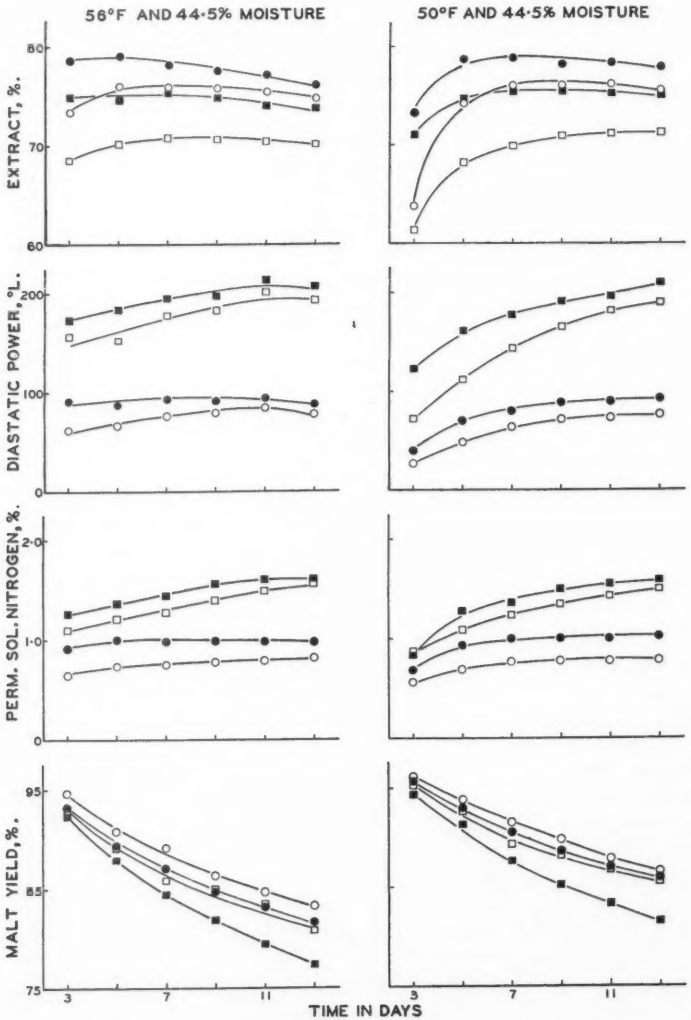

FIG. 1. *Effect of temperature and germination time on the quality of malts made from O.A.C. 21 and Wisconsin 38.* ●*O.A.C. 21, Nappan;* ○*Wisconsin 38, Nappan;* ■ *O.A.C. 21, Indian Head;* □ *Wisconsin 38, Indian Head.*

Results and Discussion

The data for extract, diastatic power, permanently soluble nitrogen as percentage of wort solids, and malt yield, the four main qualities in which the varieties differed, are presented in Figs. 1 and 2 as curves in which each quality is plotted against germination time. The four rows of graphs

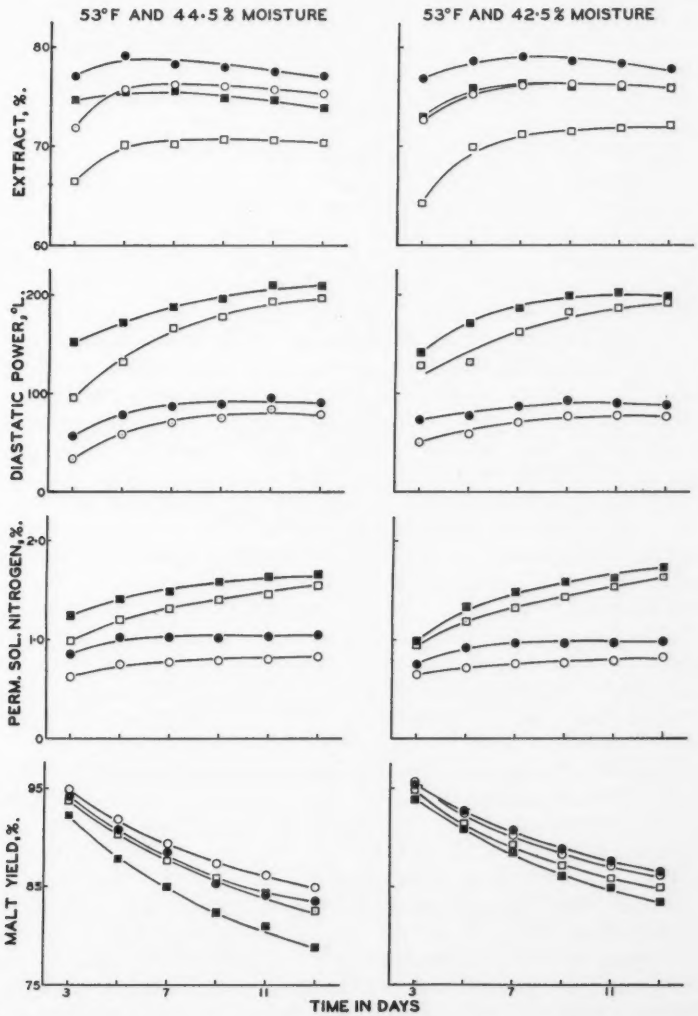

FIG. 2. *Effect of moisture content and germination time on the quality of malts made from O.A.C. 21 and Wisconsin 38.* ●*O.A.C. 21, Nappan;* ○*Wisconsin 38, Nappan;* ■ *O.A.C. 21, Indian Head;* □*Wisconsin 38, Indian Head.*

represent the four malt qualities and the four columns of graphs represent the four sets of malting conditions. The individual graphs may best be considered as two pairs of curves, each pair representing samples of the two varieties from one station.

The curves for malt yield are of interest mainly because they show that the samples grew continuously throughout the whole germination period. They also show that Wisconsin 38 grew more slowly than O.A.C. 21. The curves for the former are above the corresponding ones for the latter except for the samples from Nappan germinated with 42.5% moisture. The exception probably indicates that O.A.C. 21 is less tolerant of understeeping than Wisconsin 38.

It is apparent that for extract, diastatic power and soluble nitrogen, the curves for Wisconsin 38 fall below the corresponding ones for O.A.C. 21 and that the curves become closer with increasing time. A differential response of varieties to germination time is thus demonstrated, longer times favoring Wisconsin 38. The effect with respect to extract is attributed largely to over-modification of the O.A.C. 21, which undoubtedly took place towards the end of the germination period. This was to be expected, since this variety can usually be properly modified in about six days under conditions representing approximately the mean of those used in this investigation. When a normal degree of modification is exceeded, the decrease in soluble dry matter through respiration and root loss begins to overtake the increase resulting from the activity of the enzymes, with the result that the curves begin to flatten out, pass the maximum, and then start to fall.

The effect on the two varieties of changing the germination temperature can be observed by comparing curves in the first column of graphs in Fig. 1 with corresponding curves in the second column of graphs, and the effect of changing the moisture content can be observed by making similar comparisons between curves shown in Fig. 2. It is apparent that both responded in almost exactly the same manner to changes in these two conditions of germination. A small differential effect exists, but it is much less than the differential effect of germination time on varieties. This was probably to be expected since the range of times used was greater than the range of either temperature or moisture content.

The general effect of changing the germination conditions is illustrated more clearly by the curves in Fig. 3 which were plotted from data representing the means for all four samples. It is apparent that extract, diastatic power, and permanently soluble nitrogen all increased more rapidly at the higher temperature but that the maximum values obtained were little affected. At the lower moisture content, extract developed more slowly but attained a higher maximum value. In view of the experimental errors of the investigation, it seems doubtful whether any significance should be attached to the small difference between the curves for diastatic power. The effect of moisture content on soluble nitrogen was also small, though there is an indication that development was less rapid but attained a higher maximum value at the lower moisture content.

Piratzky and Rehberg (10), and earlier continental workers cited by them, found that, in general, lower germination temperatures produced higher extract yields and higher enzymatic activity. Our study fails to support these findings. However, it seems unwise to attach any special significance to this lack of agreement. The six-rowed barleys used in our study were

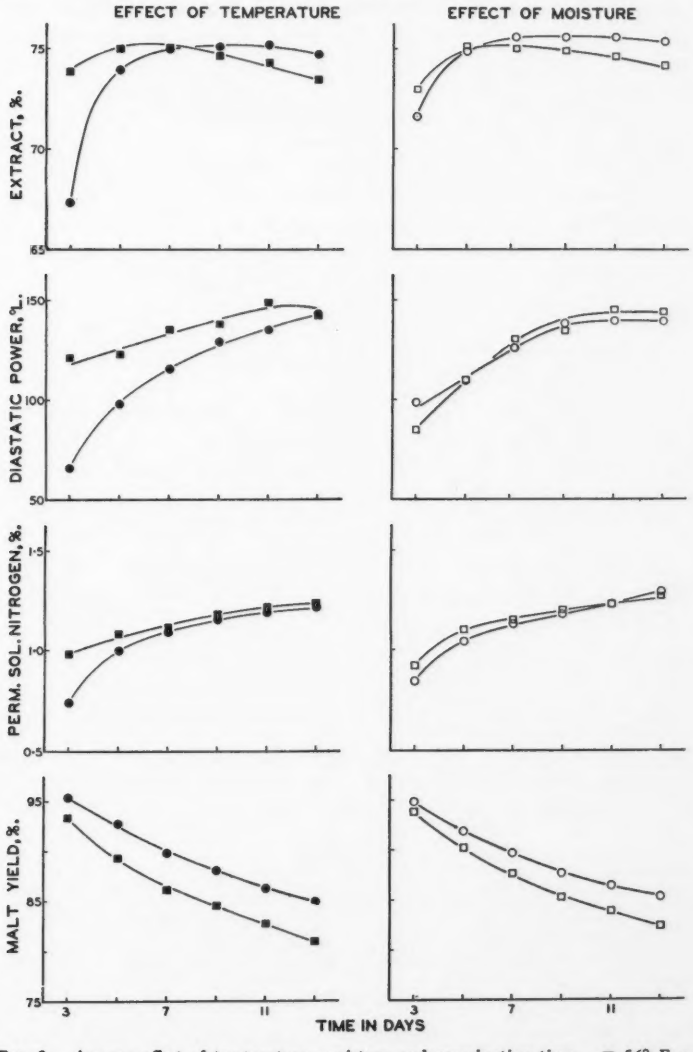

FIG. 3. *Average effect of temperature, moisture and germination time.* ■ *56° F. and 44.5% moisture.* □*44.5% moisture and 53° F.* ●*50° F. and 44.5% moisture.* ○*42.5% moisture and 53° F.*

high in nitrogen content and differ considerably in their malting qualities from the two-rowed barleys grown on the continent. Moreover, the temperatures used differed both in range and level, higher temperatures being used by continental workers in studying barleys of lower nitrogen content.

Although definite conclusions cannot be drawn from the results of a study of only two sets of samples, the data strongly suggest that there are real differences in the malting qualities of the two varieties studied, and that these differences are large in comparison with the effect on them of changing the malting conditions. It appears that the deficiencies of Wisconsin 38 cannot be overcome by such modifications of the malting method as may be considered economically practicable. With respect to extract, the evidence presented is quite clear cut. Even when the germination period is doubled, a procedure that would certainly be very uneconomical, Wisconsin 38 fails to produce as high an extract as the corresponding sample of O.A.C. 21. With respect to diastatic power and wort nitrogen, the statement must be modified, since with the Indian Head samples, increasing the germination period for Wisconsin 38 enables it to attain values as high as those given by O.A.C. 21 in a normal six-day germination period. However, when both varieties are malted in the same manner, values for O.A.C. 21 are consistently higher than corresponding values for Wisconsin 38.

We are inclined to believe that these conclusions, based mainly on the effect of increasing the germination period, would apply equally well to changes in other conditions of malting, since it seems reasonable to suppose that doubling the germination time produced greater effects than could be obtained by any reasonable modifications of steeping, sprinkling, aeration or temperature conditions.

In spite of these conclusions, it must be admitted that the curves show that the differential effect of malting conditions on varieties is appreciable, and it appears that this might well be a limiting source of error in the study of certain pairs of varieties. In these circumstances, it seems probable that the routine malting test, involving the use of one standard set of conditions, will find its greatest use in the preliminary separation of new varieties into those which are about equal in malting quality to the standard variety and those which are definitely inferior to it. More thorough investigations of the type reported in this paper will then be required for the final study of any variety which malting and agronomic data have shown to be really promising.

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THE EFFECT ON WHEAT QUALITY OF LONG EXPOSURE TO CARBON TETRACHLORIDE[1]

By R. K. Larmour[2] and H. N. Bergsteinsson[3]

Abstract

Wheat samples (5 lb.) of 12, 18 and 24% moisture content were stored at 21° C. for periods varying from 4 to 40 weeks with dosages of carbon tetrachloride from 1 to 20 cc. At 12% moisture content the wheat was not damaged by either storage or carbon tetrachloride. At 18% moisture content, samples with 10 and 20 cc. dosages of carbon tetrachloride were undamaged after 10 weeks' storage except for a slight sour odor which disappeared on drying. After 20 weeks' storage there was definite evidence of damage. Samples of 24% moisture content kept well for 4 weeks without any carbon tetrachloride, but those having the higher dosages were damaged. With longer storage all samples at 24% moisture content, no matter how treated, underwent spoilage.

Introduction

In connection with a program of investigations on the prevention of damp wheat spoilage, Larmour, Clayton and Wrenshall (2) reported that exposure of damp wheat for 25 days to sufficient carbon tetrachloride to prevent heating caused no deterioration of baking quality attributable to the chemical. They used wheat at 24% moisture content and dosages of carbon tetrachloride varying from 1 to 12 cc. per 5-lb. sample of wheat. It was observed, however, that storage at this moisture content without any carbon tetrachloride caused a marked decrease in resistance to high dosages of flour improvers such as potassium bromate, and combinations of potassium bromate, diastatic malt, and ammonium phosphate. This was not noticeable with the simple baking formula and with formulas involving dosages of potassium bromate alone up to 0.003%. Larmour (1), in an earlier study, found that storage at moisture contents up to 22% did not affect the baking quality except in cases where the wheat became moldy. As only the simple and bromate formulas were used in this earlier study, it appears probable that failure to discover damage may be attributed to the inadequacy of the baking tests used at that time. In 1934, Swanson (3) reported that damp wheat stored with the fungicide Ceresan (2% ethyl mercuric chloride in 98% inert dry material) showed no apparent damage to baking strength as a result of 4 weeks' storage, but after 13 to 16 weeks' storage there was a marked decrease in loaf volume. This indicated that our storage experiment might not have been continued long enough to make a rigorous test of the action of carbon tetrachloride on damp wheat. For these various reasons it seemed advisable to repeat the work, using both treated and untreated samples, and longer storage periods.

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Material and Treatment

The wheat used was a uniform four-bushel lot of Marquis, grown at Saskatoon in 1934. When received it had a moisture content of 11.3%, protein content of 17.5%, and was graded No. 1 Northern. The samples were stored in tin cans 7 in. high by 6 in. in diameter, fitted with slip covers which were heavily paraffined at the joints. Five pounds of wheat practically fill these cans. They were stored in a sub-basement room which is kept at 21° C. continuously by thermostatic control. The scheme of the experiment is given in Table I.

TABLE I
PLAN OF THE STORAGE EXPERIMENT

Dosage of CCl_4, cc.	Time of storage, weeks	Sample No.		
		Initial moisture of stored wheat		
		12%	18%	24%
None	4	1	2	3
None	10	13	14	15
None	20	25	26	27
None	40	37	38	39
1	4	4	5	6
1	10	16	17	18
1	20	28	29	30
1	40	40	41	42
10	4	7	8	9
10	10	19	20	21
10	20	31	32	33
10	40	43	44	45
20	4	10	11	12
20	10	22	23	24
20	20	34	35	36
20	40	46	47	48

As the appropriate cans were opened after each storage period, the samples were carefully examined for odor and appearance; samples were taken for moisture determinations, and those with 18% and 24% moisture were spread on screen-bottomed trays to dry. After drying to about 13% or lower, they were tempered and milled in the usual way, and baked after the flour had aged for one month. Two baking formulas were used:— (i) the standard malt-bromate-phosphate formula (namely, NaCl 1%, sugar 2.5%, yeast 3.0%, 200° Lintner malt 0.3%, $KBrO_3$ 0.001%, and $NH_4H_2PO_4$ 0.1%) and (ii) a high intensity formula with all ingredients the same as (i) except yeast 5% and sugar 6%. Mixing procedure, fermentation times and temperatures were as prescribed for the A.A.C.C. standard baking test.

Discussion of the Results

The baking data, together with observations on the condition of the wheat when taken from storage, are given in Tables II, III and IV. They are grouped according to moisture content of the samples.

As was expected, there was no appreciable change in wheat at 12% moisture with any of the dosages of carbon tetrachloride used.

Wheat stored at 18% moisture for 10 weeks showed no evidence of deterioration of baking quality. The check sample and the sample with 1 cc. carbon tetrachloride were slightly musty in smell; those with higher dosages were slightly sour but not musty. These odors of the wheat samples could not

be detected in the flours or in the bread. Storage for 20 weeks and longer resulted in damage to baking quality even with the smallest dosages of carbon tetrachloride.

The samples at 24% moisture, after four weeks' storage, gave no evidences of mustiness. They all had a slight sour odor which was not carried over to the flour or bread. The baking data, however, revealed that, while the check sample was undamaged, the treated ones showed distinct evidence of damage to quality. The extent of the damage was slight in the sample stored with 1 cc. of carbon tetrachloride but quite severe in the two other samples. All other samples in the 24% moisture-content series were damaged. The extent of the damage to quality increased with time, and in all cases those treated with the 10 and 20 cc. dosages deteriorated more than either the untreated check sample or the sample treated with 1 cc. of carbon tetrachloride. It is evident, therefore, that carbon tetrachloride has a deleterious effect on wheat at 24% moisture content.

Attention should be directed to Sample 15, Table IV. This sample (which was untreated) gave no evidence of mustiness, only a very slight sour odor, and was of excellent color and appearance, but it had undergone pronounced deterioration in baking quality. This damage to baking quality must be attributed to changes induced by the high moisture content. The exceptionally fine color of these high-moisture samples was in striking contrast to the pronounced bleached appearance of the samples stored at 18% moisture. No explanation of this has been found yet.

TABLE II

DATA ON WHEAT STORED AT 12% MOISTURE CONTENT

Sample No.	Time of storage, weeks	Dosage of CCl_4, cc.	Moisture after storage, %	Malt-bromate-phosphate Formula (1)		High-speed Formula (2)		Remarks on condition of wheat after storage	
				Loaf vol., cc.	Baking score	Loaf vol., cc.	Baking score	Color of wheat	Odor of CCl_4
1	4	0	12.1	960	159	980	166	No change	
4	4	1	12.2	1035	174	965	163	No change	Slight
7	4	10	12.2	1005	168	1050	180	No change	Strong
10	4	20	12.4	1040	176	950	161	No change	Very strong
13	10	0	12.2	1075	184	1150	198	No change	
16	10	1	12.3	1145	199	1125	192	No change	Slight
19	10	10	12.5	1075	184	1150	197	No change	Strong
22	10	20	12.4	1115	192	1125	192	No change	Strong
25	20	0	12.3	1035	174	1040	184	No change	
28	20	1	12.6	1085	184	1060	190	No change	None
31	20	10	12.5	1095	187	1055	186	No change	Strong
34	20	20	12.2	1008	170	1045	184	No change	None
37	40	0	12.2	1080	191			No change	
40	40	1	12.3	1150	204			No change	None
43	40	10	12.2	1090	192			No change	Slight
46	40	20	12.2	940	163			No change	Slight

TABLE III

DATA ON WHEAT STORED AT 18% MOISTURE CONTENT

Sample No.	Time of storage, weeks	Dosage of CCl_4, cc.	Moisture after storage, %	Malt-bromate-phosphate Formula (1)		High-speed Formula (2)		Remarks on condition of wheat after storage			
				Loat vol., cc.	Baking score	Loaf vol., cc.	Baking score	Color of wheat	Odor of CCl_4	Musty odor	Sour odor
2	4	0	18.2	1000	167	910	152	Slightly bleached	–	Slight	–
5	4	1	18.4	1025	174	955	161	Slightly bleached	Slight	Slight	–
8	4	10	17.9	905	148	960	162	Slightly bleached	Strong	Nil	Nil
11	4	20	17.9	935	157	900	150	Slightly bleached	Strong	Nil	Nil
14	10	0	18.6	1025	175	1010	170	Quite bleached	–	Slight	Nil
17	10	1	18.6	1075	184	1035	174	Quite bleached	Nil	Slight	Nil
20	10	10	18.2	1010	170	1070	180	Quite bleached	Slight	Nil	Slight
23	10	20	17.8	1030	175	1005	168	Slightly bleached	Slight	Nil	Slight
26	20	0	18.2	905	149	880	151	Bleached	–	Slight	Very slight
29	20	1	18.2	940	157	890	152	Bleached	Nil	Slight	Nil
32	20	10	17.8	932	155	835	139	Bleached	Nil	Nil	Slight
35	20	20	17.0	885	145	873	147	Bleached	Nil	Slight	Slight
38	40	0	18.7	820	125			Bleached	–	Slight	–
41	40	1	19.1	895	146			Bleached	Nil	Marked	Marked
44	40	10	18.4	870	147			Slightly bleached	Slight	Slight	Marked
47	40	20	18.3	785	126			Bleached	Nil	Slight	Slight

TABLE IV

DATA ON WHEAT STORED AT 24% MOISTURE CONTENT

Sample No.	Time of storage, weeks	Dosage of CCl_4, cc.	Moisture after storage, %	Malt-bromate-phosphate Formula (1)		High-speed Formula (2)		Remarks on condition of wheat after storage			
				Loaf vol., cc.	Baking score	Loaf vol., cc.	Baking score	Color of wheat	Odor of CCl_4	Musty odor	Sour odor
3	4	0	24.1	995	166	945	156	Slightly bleached	–	Nil	Slight
6	4	1	23.9	950	157	870	141	Slightly bleached	Nil	Nil	Slight
9	4	10	24.0	795	126	710	110	Good	Slight	Nil	Slight
12	4	20	23.7	765	126	620	91	Good	Marked	Nil	Slight
15	10	0	24.4	800	120	600	75	Good	–	Nil	Slight
18	10	1	24.4	860	131	750	111	Good	Very slight	Very slight	Slight
21	10	10	24.1	625	79	615	82	Good	Slight	Nil	Slight
24	10	20	24.5	575	68	520	55	Good	Marked	Nil	Slight
27	20	0	25.0	648	68	578	49	Slightly bleached	–	Slight	Marked
30	20	1	24.8	840	104	775	89	Slightly bleached	Nil	Marked	Marked
33	20	10	23.8	470	25	440	15	Excellent	Nil	Slight	Marked
36	20	20	24.3	415	13	435	14	Excellent	Marked	Slight	Marked
39	40	0	25.4	440	15			Bleached	–	Very musty	Very sour
42	40	1	25.0	425	16			Slightly bleached	Nil	Slight	Marked
45	40	10	24.7	360	2			Excellent	Nil	Slight	Marked
48	40	20	25.6	357	−2			Excellent	Slight	–	Marked

The baking data given in Tables II, III and IV were obtained at different times and therefore are subject to rather large errors, if one wishes to compare the results of the various storage periods. In order to get more comparable data, all the samples of flour from the preceding series were rebaked by Formula 1 at the time of baking the 40-week series. The results are given in Table V. There is, of course, another source of variation in this series of values, namely, the aging of the earlier-milled flours. There is no way of knowing whether the keeping quality of some of the flour samples had been damaged by the treatment of the wheat, but if one compares these values with those obtained in the earlier bakings, there is found little evidence of deterioration due to storage as flour. From the data of the final baking in Table V, the following conclusions seem to be warranted: (i) Dry wheat suffers

TABLE V

LOAF VOLUMES OBTAINED IN THE FINAL BAKING OF THE FLOURS BY THE MALT-BROMATE-PHOSPHATE FORMULA

Dosage of CCl_4, cc.	Time of storage as wheat, weeks	Time of storage as flour, weeks	Loaf volumes			Loaf volume — first baking — after flour was stored 4 weeks		
			12%	18%	24%	12%	18%	24%
0	4	40	1040	920	947	960	1000	995
1	4	40	1095	1065	975	1035	1025	950
10	4	40	985	1070	780	1005	905	795
20	4	40	1062	965	790	1040	935	765
0	10	34	985	915	815	1075	1025	800
1	10	34	1080	942	886	1145	1075	800
10	10	34	1025	995	600	1075	1010	625
20	10	34	1105	967	490	1115	1030	575
0	20	24	1110	910	672	1035	905	648
1	20	24	1150	910	810	1085	940	840
10	20	24	1015	945	380	1095	932	470
20	20	24	1040	930	380	1008	885	415
0	40	4	1080	820	440			
1	40	4	1150	895	425			
10	40	4	1090	870	360			
20	40	4	940	785	357			

no damage from exposure to carbon tetrachloride over a period of 40 weeks. (ii) Wheat at 18% moisture content shows some deterioration as a result of storage for 40 weeks. This cannot be attributed to the effect of carbon tetrachloride, but rather to the mustiness and sourness developed. (iii) Wheat at 24% moisture content suffered no damage when stored 4 weeks without carbon tetrachloride or any other fungicide and only slight damage when stored with 1 cc., but with dosages of 10 and 20 cc. there was marked evidence of damage attributable to the carbon tetrachloride. After 10 weeks' storage, the check sample, although showing no marked physical damage, gave distinct evidence of deterioration. This could be attributed only to the high moisture

content. The effect of carbon tetrachloride was more pronounced than after 4 weeks' storage. After 20 weeks' storage all samples were heavily damaged. (iv) The baking behavior of some of the more severely treated samples indicates that the high-speed baking formula may prove useful in accentuating quality differences where damage is likely to have occurred.

These results are similar to Swanson's (3) conclusions concerning the action of Ceresan and indicate that with very damp wheat, carbon tetrachloride should be used only for relatively short periods. The four-week period seems safe enough with sound wheat at moisture contents as high as 24%; this should generally be sufficient time to move the grain into a drier, where the removal of moisture would prevent any damage from carbon tetrachloride remaining in the grain.

It is evident that in this study there are two effects which require further investigation. The more important of these is the apparent deterioration occurring when wheat is kept at a high moisture content. In the experiment reported the samples were stored at 21° C., and even at that relatively high temperature those at 24% moisture content were practically sound after 10 weeks' storage in sealed containers. At lower temperatures the storage periods might be extended without causing spoilage. All properties of the wheat that can be estimated with fair accuracy should be investigated in order to find, if possible, what changes occur under these conditions.

The other problem is concerned with the rapid disappearance of carbon tetrachloride in wet grain and the accompanying production of sourness, which may be the result of catalytic hydrolysis, with the production of hydrochloric acid and carbon dioxide. This can be tested readily and should be of academic, if not of practical, interest.

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OBSERVATIONS ON THE STUDY OF VARIETAL DIFFERENCES IN THE MALTING QUALITY OF BARLEY. PART III[1]

By J. Ansel Anderson[2] and W. O. S. Meredith[3]

Abstract

Samples of eight barley varieties grown at six widely separated points in Canada were malted in duplicate under standard conditions in laboratory equipment. After six days in the germinator, half of each sample was removed and kilned. The remaining halves were grown two days longer before kilning. The relative positions of the varieties with respect to extract, diastatic power, and permanently soluble nitrogen, were changed by the additional two days' growth, but the changes were generally small by comparison with the spreads between varieties and the greater changes in their relative positions when grown at different stations. It is concluded that the differential effect of malting method is an appreciable source of error in the interpretation of the results of routine malting tests, but that the limiting factor in studies of the comparative malting qualities of varieties is the differential effect of environment on them.

In Part II of these studies (2) the differential effect of malting method on barley varieties was investigated, using six germination times and four sets of malting conditions, but samples of only two varieties from two stations. The present study was undertaken concurrently and is of the same type. In a sense, it is complementary to the preceding one since samples of eight barley varieties from six stations were used, but the malting conditions represented germination for six and eight days only. These limitations were imposed because the study was carried out, as an extension of an ordinary variety trial, in the laboratory in which routine malting tests are made for Canadian plant breeders. The six-day germination period represented the standard conditions required for the original trial. The eight-day period was chosen as representing a reasonable change in malting procedure and one which could be used with little disruption of the routine of the laboratory.

Materials and Methods

Samples of the six-rowed, rough-awned varieties, O.A.C. 21, Olli, Peatland, and Pontiac; the six-rowed, smooth-awned varieties, Nobarb, Regal, and Velvet; and the two-rowed, smooth-awned variety, Rex; were obtained from the following stations: Beaverlodge and Edmonton, Alberta; Melfort, Saskatchewan; Brandon and Gilbert Plains, Manitoba; and Ste. Anne de la Pocatière, Quebec. The varieties differ widely in their malting characteristics and represent a good cross-section of the materials submitted for malting tests in Canada.

The barleys were grown in plots of five rod-rows arranged in a modified balanced block with quadruplicate plots for each variety. Marginal effects

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were reduced by harvesting only the centre three rows, and samples for malting were obtained by bulking the grain from quadruplicate plots. The eight varieties were selected from a total of twenty-four which were being grown mainly for the study of agronomic characters. It is apparent that under these conditions field errors resulting from soil heterogeneity will probably be somewhat greater than they would have been if the eight varieties used in this study had been grown in smaller blocks.

The six-day malts were made and analyzed at the University of Manitoba, using laboratory malting equipment and methods which have been described previously (1). After the samples had been in the germinator for six days, half of each was removed and kilned. The remaining halves were sprayed with 15 gm. of water and grown for an additional two days before kilning.

Results and Discussion

Since the magnitude of the differential response of the varieties to the change in malting conditions may be expected to depend on the magnitude of the average effect of the change on all varieties, this must be examined first. The required data are given at the top of Table I as means, over all varieties, for six- and eight-day malts. The additional two days in the germination chamber resulted in an average increase of 0.2 in percentage extract, 13° Lintner in diastatic power, 0.17 in permanently soluble nitrogen as percentage of wort solids, and 3.5 in percentage malting loss. It is therefore apparent that considerable growth and modification took place during the extra two days, and that opportunity was provided for varieties which grow and modify slowly to show to better advantage in the eight-day series.

The second line of figures in Table I gives the differences between variety means which can be considered statistically significant (five per cent level) in view of the differential response of varieties to environment, which appears to be a limiting factor in investigations of varietal differences in malting quality. (References on this point were listed in Part II of this series, 2.) It may be assumed that varieties whose means differ by less than the amounts listed will fall in different orders at different stations, and must therefore be considered about equal with respect to the malt quality in question. On the other hand, when the means for two varieties differ by the required amount, the odds are 19 to 1 that a real difference between the varieties is operating to spread the means. In such cases the variety having the higher mean will generally be found to yield higher values at all, or almost all stations, and for all practical purposes the varieties must therefore be considered to differ in malting quality.

The main data for the investigation are presented in summarized form, as varietal means over all stations, in the body of Table I. The varieties are listed in each column in descending order with respect to the malt quality in question. To facilitate examination of the data, only the names of those varieties which changed their positions have been listed in the columns for eight-day malts.

TABLE I

MEAN VALUES FOR SIX- AND EIGHT-DAY MALTS

Extract, %				Diastatic power, °L.				Perm. sol. nitrogen as % of wort solids				Malting loss, %			
6-day		8-day		6-day		8-day		6-day		8-day		6-day		8-day	
Mean values over all samples															
72.8		73.0		142		155		1.16		1.33		7.6		11.1	
Necessary differences required for a 5% level of significance															
1.2		1.3		15		16		0.10		0.11		0.6		0.8	
Mean values, over all stations, for each variety															
Olli	75.3		75.4	Olli	187		206	Olli	1.34		1.51	Olli	8.6		12.1
O.A.C. 21	73.5	Rex	74.0	Peatland	158		175	Rex	1.24		1.44	O.A.C. 21	8.0	Rex	11.5
Rex	73.4	O.A.C.21	73.4	Pontiac	151	Rex	161	Peatland	1.21	Velvet	1.39	Pontiac	8.0	O.A.C.21	11.4
Pontiac	72.7		72.8	Rex	145	Pontiac	158	Velvet	1.16	Peatland	1.38	Peatland	7.6	Velvet	11.1
Nobarb	72.4		72.7	O.A.C.21	145	Velvet	154	O.A.C.21	1.14		1.27	Rex	7.4	Pontiac	11.0
Peatland	72.2		72.3	Velvet	138	O.A.C.21	152	Pontiac	1.11		1.27	Velvet	7.3	Peatland	11.0
Regal	71.6		71.7	Nobarb	117		134	Regal	1.07		1.19	Regal	7.3		10.7
Velvet	71.2		71.6	Regal	96		103	Nobarb	0.96		1.15	Nobarb	6.8		10.1

While the data show several changes of varietal position between the six- and eight-day series, in only one instance is the change significant. Thus, with respect to extract, O.A.C. 21 and Rex reversed their positions, but the difference of 0.6% between them cannot be considered significant. Two pairs of varieties, Pontiac and Rex, and O.A.C. 21 and Velvet, reversed their positions with respect to diastatic power, but neither pair can be considered to differ significantly in either series. In permanently soluble nitrogen, Velvet and Peatland changed positions, but the differences between them are only half the required amount. Five varieties were involved in a change of order with respect to malting loss. Of these, Rex and Pontiac changed their relative position most and present the exception mentioned in the first sentence of this paragraph. The malting loss for Pontiac was just significantly higher than that for Rex in the six-day series, whereas in the eight-day series Rex had the higher malting loss. It is apparent that the two-rowed variety Rex did not grow as rapidly as some of the six-rowed varieties during the initial germination period but continued to grow more vigorously during the extra two days. This hypothesis is supported further by the data for extract.

Besides considering complete reversals of positions, it is also necessary to consider changes in the spreads between varieties which do not involve a reversal. These latter changes can be observed more readily by reference to Table II. The data represent differences between the mean increase over all varieties from six- to eight-day malts and the increase for the variety listed in the first column. A positive sign indicates that the value for the variety in question increased more than the average amount, and a negative sign indicates that it increased less. Thus, in any column, the varieties represented by the greatest positive and negative values changed their relative positions most.

TABLE II

DIFFERENCES BETWEEN AVERAGE INCREASE OVER ALL VARIETIES BETWEEN SIX- AND EIGHT-DAY MALTS AND INCREASES FOR INDIVIDUAL VARIETIES

Variety	Extract, %	Diastatic power, °L.	Perm. sol. nitrogen	Malting loss, %
Olli	−0.1	+ 6	0	+0.1
O.A.C. 21	−0.3	− 6	−0.05	0
Rex	+0.4	+ 3	+0.03	+0.6
Pontiac	−0.1	− 6	−0.01	−0.4
Nobarb	+0.1	+ 4	+0.02	−0.2
Peatland	−0.1	+ 4	0	−0.1
Regal	−0.1	− 5	−0.05	−0.1
Velvet	+0.2	+ 3	+0.06	+0.3
Mean increase over all varieties	+0.2	+13	+0.17	+3.5

It turns out that with respect to extract, the varieties which changed their relative position most were those which also reversed their positions, namely, O.A.C. 21 and Rex, and these cannot be considered to differ significantly.

In diastatic power, the difference between Olli and O.A.C. 21, and between Olli and Pontiac, increased by 12° Lintner, an amount which approaches the order of magnitude of the necessary difference based on the over-all differential effect of environment on varieties. With respect to permanently soluble nitrogen, the greatest change in relative positions occurred with Velvet on the one hand and O.A.C. 21 and Regal on the other. The increase in the difference amounts to 0.11, which equals the necessary difference. The data for malting loss show that the greatest change in position also led to a reversal in position. It concerns Rex and Pontiac and was discussed above.

The results of the study again suggest that the differential effect of malting method on varieties is not as large as the differential effect of environment. The former, nevertheless, constitutes an appreciable source of error in the interpretation of the results of routine malting tests. As a result, it is apparent that supplementary investigation of all reasonably promising varieties will be required before an adequate estimate of their malting qualities can be obtained.

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STUDIES ON FOOT- AND ROOT-ROT OF WHEAT

VI. METHODS OF SECURING INFECTION OF WHEAT SEEDLINGS FOR STUDY IN NUTRIENT SOLUTIONS[1]

By W. C. Broadfoot[2] and L. E. Tyner[3]

Abstract

The two foot-rot diseases of wheat caused by *Helminthosporium sativum* P. K. & B. and *Fusarium culmorum* W. G. Sm. were studied in nutrient culture solutions instead of in the usual substrates of soil or sand. The most satisfactory results were obtained by first germinating the grains in a specially designed tray, then securing infection of the young plants by adding inoculum to the tray, after which the seedlings were transplanted to the nutrient culture solution. Infection of the seedlings was distinctly increased when sucrose was added to a nutrient solution infested previous to the time of transplantation. Infection was less satisfactory when the seed was immersed in a spore suspension, dried, and germinated on the tray. Very unsatisfactory infection was secured by adding a spore suspension in water, with or without sugar, to the nutrient solution at the time of transplanting the seedlings. Inoculating the seedlings with a spore suspension by means of a hypodermic needle produced practically no infection. Length of shoot, and particularly the dry and the green weight of the entire plant were reliable quantitative criteria for the evaluation of disease. The first method indicated appears to offer several important advantages in that the degree of infection can be controlled.

It is well known that in the study of the foot-rot disease of cereals, certain very important difficulties in technique have seriously hampered progress. By present methods of artificial inoculation, where concentrated inoculum of the pathogen is placed around or near the grain, the grain itself is often destroyed before it can germinate. If germination occurs, the young seedling may be killed before or soon after emerging from the ground. Should the seedlings survive this ordeal, they are often so severely crippled that a proper study of the natural resistance of the host to the disease becomes practically impossible. On the other hand, if the inoculum is not in direct contact with the grain as it germinates, many plants may escape infection, and thus a disconcerting experimental variable is introduced. If satisfactory infection of the seedlings could be secured by introducing inoculum to the soil at a later period, the problem would be simplified; but on account of the antagonistic action of the soil flora to the pathogen (3, 5), and other associated difficulties of technique, this does not give the desired results.

The purpose of this study was to find a suitable method of producing a uniform but not too severe infection of the seedlings after the grains germinate, so that these difficulties would be avoided.

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Materials and Methods

Infection Technique

Marquis wheat seed was surface disinfected in a 1-1000 mercuric chloride solution for 20 min., washed in running tap water for 60 min., soaked overnight in distilled water at 24° C., and then germinated in special trays. These trays were, with certain slight modifications, essentially the same as used by Hoagland and Broyer (4). The seed was distributed on cheesecloth on a cedar frame, 20 × 40 cm., which was placed in a photographic tray. A similarly covered, but smaller, frame was then placed over the first, about 1 cm. above the seed level. The edges of the cheesecloth on both frames dipped into the water in the bottom of the tray. During germination at 20° C., all trays were covered with glass sheets and brown wrapping paper. Several methods of securing infection were tried. These will be described later in connection with each experiment. Spore suspensions of *Helminthosporium sativum* P. K. & B. and of *Fusarium culmorum* W. G. Sm. were used as pathogens.

Nutrient Solution Technique

The plants were grown in nutrient solutions instead of the usual substrates of soil or sand. Two types of containers were employed, *viz.*, large iron tanks 30 × 30 × 8 in., of 115 litre capacity, coated with asphalt paint, described by Arnon (1), and the ordinary glazed earthenware, one-gallon crocks. The covers of the tanks held 64 corks, each supporting one plant. The sheet metal tops of the crocks, coated with paraffin wax, held five corks, each supporting one plant.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS IN CC. PER LITRE

No.	Nutrient solution	$Ca(NO_3)_2$ M/1	KNO_3 M/1	$MgSO_4$ M/1	KH_2PO_4 M/1	K_2SO_4 M/.5	$Ca(H_2PO_4)_2$ M/.01	$CaCl_2$ M/1
1	Complete	5	5	2	1			
2	Low potassium	6.75	1.5	2			50	
3	High potassium	5	5	2	1	9		
4	Low nitrogen	1.25	1.25	2		8.75	50	
5	High nitrogen	8.75	8.75	2	1			
6	Low calcium	1.25	10	2	1			
7	High calcium	5	5	2				3.75

The nutrient culture solutions were varied according to need from basic formulas which were kindly furnished by Professor D. R. Hoagland of the University of California. The compositions of the nutrient solutions employed in all experiments reported are given in Table I. The nutrients were added to the tanks and to the crocks in amounts proportional to 115 and 4 litres, respectively. Iron, as ferric tartrate, at the rate of 1 cc. of 0.5% solution per litre, was added once a week in Experiments I, II, III and IV, and twice a week in Experiments V and VI. The pH of the nutrient solutions was also determined colorimetrically.

Other Technique

While the experiments were in progress, four 500-watt lights, suspended three feet above the greenhouse bench, were turned on from about 4 to 12 p.m.

each day. The plants were taken up approximately 40 days after they were transplanted. At this time, the length of shoots and roots, and their green weight were recorded. The plants were then dried in an oven at 90° C. for 48 hr., and weighed. Where possible, the experimental data were tested by Fisher's (2) Analysis of Variance method, and by the "F" test of Snedecor (6) to determine the significance of the differences observed among the various treatments. Other necessary details of technique will be supplied in conjunction with each experiment.

Experimental Results

The methods of securing infection which proved unsatisfactory were: *(a)* a spore suspension of the pathogen injected into the crown area of the seedlings by means of a hypodermic needle; *(b)* a spore suspension of the pathogen in water, or *(c)* in sucrose solution, added to the nutrient solution in the tanks at the time of transplanting the seedlings; *(d)* the seed soaked in a spore suspension of the pathogen prior to its germination. The results from the four methods indicated are reported in Experiments I, II, III and IV, respectively.

The methods which gave satisfactory infection were: *(a)* a spore suspension of the pathogen, plus sugar, added to the nutrient solution in the tanks some time prior to transplanting the seedlings; *(b)* the roots of the seedlings immersed in the inoculum prior to transplanting them to the tanks. The results from these two methods are reported in Experiments V and VI, respectively.

Inoculation with Hypodermic Needle (Experiment I)

On October 25, 1935, Marquis wheat grains were spread on trays to germinate. Five days later, 64 uniform seedlings were transplanted to each of the six 115-litre tanks, previously described. Two tanks contained a complete nutrient solution, two others a high-calcium solution, and the remainder a low-calcium solution. The compositions of the nutrient solutions are given in Table I, Nos. 1, 6 and 7. Eleven days later, 1 cc. of a spore suspension of *H. sativum* was injected with a hypodermic needle into the crown tissue of the plants growing in one of each pair of tanks indicated above. Similar amounts of sterile distilled water were injected into the crowns of the plants growing in each of the three corresponding control tanks. This experiment was taken up on December 6. The results are given in Table II.

TABLE II

EFFECT ON DISEASE EXPRESSION* OF A SPORE SUSPENSION OF *Helminthosporium sativum* INJECTED HYPODERMICALLY INTO THE CROWNS OF WHEAT SEEDLINGS

Nutrient solution	pH of solution		Shoot length, cm.		Root length, cm.		Total length, cm.		Dry weight, mg.	
	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control
Complete	7.1	7.1	36.4	37.4	29.9	38.3	66.3	75.7	328.1	364.1
Low calcium	6.5	6.5	43.1	42.6	42.8	45.3	85.9	88.0	437.5	532.8
High calcium	7.1	7.0	41.0	38.1	33.1	38.3	74.0	76.4	400.0	375.0

* *In terms of plant growth.*

These indicate that a very slight amount of disease developed in plants in any of the nutrients. Therefore, this method of securing infection was ineffective. The length of roots and length of the plants in the inoculated series were a little less than in the control series of the corresponding nutrient solution, but there was no difference in length of shoots. However, the dry weight of the plants in the complete solution and in the low-calcium solution was respectively 10 and 18% less than the dry weight of the plants in the corresponding control tanks.

Inoculation by Adding Spore Suspension, without Sugar, to Tank Solution (Experiment II)

This experiment, which was begun December 10, 1935, and harvested January 20, 1936, was carried out in the six 115-litre tanks used in Experiment I. The complete nutrient solution was the same as in that experiment, but instead of low- and high-calcium solutions, low- and high-nitrogen solutions were substituted. The composition of these solutions is indicated in Table I, Nos. 1, 4 and 5.

Marquis wheat seedlings of uniform size were transplanted to all tanks. These seedlings were from seed set to germinate five days before. The inoculum consisted of a heavy spore suspension from colonies of *H. sativum* 30 days old. One tank of each nutrient solution pair received inoculum, the control did not.

The results, which are given in Table III, indicate that the disease did not develop satisfactorily in any of the solutions. On the contrary, the plants in all three inoculated tanks were decidedly greener, and had longer shoots and roots than the plants which grew in the control tanks. The dry weight of the plants grown in the high-nitrogen solution plus pathogen was 10% less than that in its control. In the other two solutions, there was no difference in dry weight of plants in the infested and control series.

TABLE III

EFFECT ON DISEASE EXPRESSION* OF ADDING A SPORE SUSPENSION OF *Helminthosporium sativum*, WITHOUT SUGAR, TO NUTRIENT SOLUTIONS AT TIME OF TRANSPLANTING WHEAT SEEDLINGS

Nutrient solution	pH of solution		Shoot length, cm.		Root length, cm.		Total length, cm.		Dry weight, mg.	
	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
Complete	7.1	7.1	30.8	27.9	27.5	26.1	58.3	54.1	173.4	175.0
Low nitrogen	6.5	6.7	49.5	46.6	51.7	42.1	101.2	88.7	437.5	418.8
High nitrogen	7.0	7.1	39.0	35.5	44.6	41.1	83.6	76.6	276.6	309.4

* *In terms of plant growth.*

Inoculation by Adding Spore Suspension with Sugar to Tank Solution (Experiment III)

This experiment, which ran from January 25 to February 14, 1936, was similar in plan to Experiment II, with the exception that the solutions were

high or low in potassium rather than nitrogen, and that all tanks received, in addition to the regular nutrient, equal amounts of sugar. The compositions of the nutrient solutions are given in Table I, Nos. 1, 2 and 3.

Uniform seedlings were transplanted to the six tanks five days from the time the seed was placed on the trays to germinate. At this time the pathogen, in the form of a spore suspension, was added to one series of solutions and sugar was added to all tanks. The sugar concentration of each tank was approximately 0.01%. The results of this test are given in Table IV.

TABLE IV

EFFECT ON DISEASE EXPRESSION* OF ADDING A SPORE SUSPENSION OF *Helminthosporium sativum*, WITH SUGAR, TO NUTRIENT SOLUTIONS AT TIME OF TRANSPLANTING WHEAT SEEDLINGS

Nutrient solution	pH of solution		Shoot length, cm.		Root length, cm.		Total length, cm.		Dry weight, mg.	
	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
Complete	6.7	6.7	63.9	53.1	53.6	47.3	117.4	100.4	1539.1	1156.3
Low potassium	6.5	6.5	61.5	59.8	48.1	47.0	109.6	106.8	1738.1	1642.9
High potassium	6.7	6.7	54.9	39.3	34.4	44.6	89.2	83.9	1309.5	444.4

**In terms of plant growth.*

As in Experiment II, the development of the disease was very light throughout, and, therefore unsatisfactory. However, the dried plants in the complete, low-potassium, and high-potassium solutions with pathogen, weighed respectively, 33, 6 and 195% more, and were definitely taller than the plants from the control series.

Seed Soaked in Spore Suspension of H. sativum prior to Germination (Experiment IV)

This experiment lasted from October 13 to November 19, 1936. The containers used were one-gallon crocks. The complete nutrient solution as employed in Experiments I, II, and III was used as a growth medium.

Seeds of Marquis wheat were disinfected in 1 : 1000 mercuric chloride solution for 20 min., and rinsed for 30 min. in sterile distilled water. One portion of this seed was soaked for 24 hr. in a heavy spore suspension of *H. sativum* 30 days old, and the control portion was soaked for 24 hr. in sterile distilled water. Each lot was then suspended in a desiccator over calcium chloride crystals, dried for five days, and spread to germinate on trays. Seven days later, and each day thereafter for three days, uniform seedlings from each lot were transplanted to the six replicate crocks, five seedlings in each. The data from this study are given in Table V.

For the inoculated series, the "F" value exceeds the 1% point for length of shoot, and the 5% point for total length of shoot and roots. It is also significant for both green and dry weight of the plants. On the other hand, the development of disease was not very satisfactory, as apparently certain seedlings on the tray escaped infection, some were killed outright, while others

developed only a slight amount of disease. Thus, although distinctly better results were obtained by this method than by the other three methods, the technique obviously does not favor a sufficiently uniform infection to recommend its use when the plants are grown in nutrient solution.

TABLE V

EFFECT ON DISEASE EXPRESSION* OF SOAKING WHEAT SEED IN A SPORE SUSPENSION OF *Helminthosporium sativum* AND TRANSPLANTING THE SEEDLINGS TO COMPLETE NUTRIENT SOLUTION AT VARIOUS INTERVALS

Interval, days	Shoot length, cm.		Root length, cm.		Total length, cm.		Green weight†, gm.		Dry weight†, gm.	
	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control
7	32.9	34.7	30.4	31.9	63.3	66.6	11.6‡	17.3	1.16‡	1.65
8	31.5‡	35.7	31.0	32.6	62.5‡	68.4	10.9‡	15.3	1.06‡	1.47
9	31.1	33.5	32.6	33.1	63.7	66.7	11.1‡	15.0	1.17‡	1.50
10	32.5	31.7	35.1	36.9	67.6	68.6	14.2‡	17.3	1.33‡	1.63
$2 \times \sqrt{2} \times$ S.E.	2.7		4.5		5.4		2.7		.23	
"F" Inoculations	8.35		1.49		5.88		41.21		43.62	
"F" Intervals	1.78		3.66		1.17		2.78		2.53	
"F" Replicates	1.03		2.37		2.70		1.88		.84	

The 5% points for inoculations, intervals, and replicates are 4.08, 2.84, and 2.45, respectively; and the 1% points are 7.31, 4.31, and 3.51, respectively.

* *In terms of plant growth.*

† *Average weight of five plants.*

‡ *Indicates significant values between inoculated and control series.*

Inoculum, plus Sugar, Added to the Nutrient Solution Prior to Transplanting Seedlings (Experiment V)

This experiment lasted from March 19 to April 8, 1936. The six 115-litre tanks used in Experiments I, II and III were employed in this study. A nutrient solution with a high concentration of potassium was used in all tanks, because of the extra vigor which this solution plus the pathogen had given to the plants in Experiment III. Sufficient sugar was added to the solution in one pair of tanks to make the concentration 0.1%, and that of the second pair 0.3%. Sugar was not added to the third pair.

Two days prior to transplanting the seedlings to the tanks, equal amounts of a spore suspension of *H. sativum* were added to one unit of each pair. This suspension was the same as used in all previous experiments, that is, spores from colonies 30 days old, grown in ten large test tubes. The seedlings were transplanted, 64 per tank, seven days after the seed was set to germinate on trays. The results of this study are presented in Table VI.

Fifteen days after transplantation, the plants became distinctly chlorotic in the control tank which received no sugar and no inoculum. After the eighteenth day, ferric tartrate was added twice each week to the solution in all tanks, instead of once each week, as had been done in all previous experiments. Within three days the chlorosis began to disappear. But the point of interest is that at the conclusion of this experiment, in the unit to which the pathogen was added, the lengths of the shoots, of the roots, and of the whole plants were about 10, 14, and 13%, respectively, longer than for

the plants in the corresponding control units. In contrast to these results, in the other two series to which pathogen was added, but with 0.1 and 0.3% of sugar, respectively, the plants were distinctly shorter in length of shoot and of root, and the dry weight was much less than that in the corresponding control series. In other words, the addition of sugar to the nutrient solution promoted infection by the pathogen, and thereby increased the development of the disease.

TABLE VI

EFFECT ON DISEASE EXPRESSION* OF ADDING A SPORE SUSPENSION OF *Helminthosporium sativum*, PLUS VARIOUS CONCENTRATIONS OF SUGAR, TO A HIGH-POTASSIUM SOLUTION, TWO DAYS PRIOR TO TRANSPLANTING WHEAT SEEDLINGS

Sugar, %	pH of solution		Shoot length, cm.		Root length, cm.		Total length, cm.		Dry weight, mg.	
	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
0	6.7	6.8	36.1	32.6	44.8	39.2	80.9	71.7	509.4	559.4
0.1	6.6	6.7	26.2	37.4	8.0	19.9	34.2	57.3	192.2	625.0
0.3	6.6	6.7	20.0	25.6	5.9	9.2	25.9	34.8	94.7	165.6

* *In terms of plant growth.*

Inoculation of Roots of Seedlings Prior to Transplanting (Experiment VI)

The duration of this experiment was from October 1, 1936, to November 4, 1936. One-gallon crocks were used. These were filled with the complete nutrient solution, without the addition of other minerals, sugar, or a spore suspension of the pathogen. Infection of the seedlings was secured by immersing their roots for various periods in inoculum of *H. sativum*. This inoculum was prepared by incubating the pathogen for ten days in a complete nutrient solution to which a 2% solution of sugar was added. Five days after the seed was set to germinate on trays, the roots of the seedlings were placed in the inoculum. At the end of seven days, and also each day thereafter for six days, uniformly infected seedlings were transplanted to the six replicate crocks, five seedlings in each. The seedlings for the corresponding controls were germinated on another tray at the same time, and the roots were immersed in the nutrient solution for the same intervals as given the corresponding inoculated units. The seedlings were then transplanted to the control crocks. The results are presented in Table VII.

The "F" value for length of shoot, length of roots, total length of shoot and roots, green weight, and dry weight of the plants indicated very significant differences for the various inoculation periods. In other words, the severity of the disease depended upon the time the roots of the seedlings were immersed in the inoculum. Thus, by suitable manipulation of the stage at which the roots are placed in contact with the pathogen, and the duration of the contact, it appears that the severity of the subsequent development of the disease can be reduced or increased as desired.

The foregoing technique was employed in testing the disease reaction of wheat seedlings to *Fusarium culmorum*, with essentially the same results as have just been reported for *H. sativum*.

TABLE VII

EFFECT ON DISEASE EXPRESSION* OF IMMERSING ROOTS OF WHEAT SEEDLINGS IN INOCULUM OF *Helminthosporium sativum* FOR VARIOUS INTERVALS, THEN TRANSPLANTING TO COMPLETE NUTRIENT SOLUTION

Interval, days	Shoot length, cm.		Root length, cm.		Total length, cm.		Green weight†, gm.		Dry weight†, gm.	
	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control
7	28.3‡	34.0	34.8‡	41.3	63.1‡	75.3	9.9‡	17.6	.89‡	1.64
8	28.7‡	33.8	34.4‡	42.0	63.1‡	75.8	9.6‡	15.9	.84‡	1.46
9	28.9‡	33.1	33.1‡	39.0	62.0‡	72.1	9.1‡	15.3	.83‡	1.37
10	27.3‡	32.2	32.1	36.2	59.4‡	68.3	7.5‡	11.3	.72‡	1.02
11	25.8‡	30.3	28.0‡	34.7	53.7‡	65.3	5.7‡	11.7	.55‡	.99
12	23.5‡	30.4	25.6‡	31.2	49.0‡	61.6	5.9‡	12.0	.49‡	1.12
13	24.7‡	30.9	24.6‡	33.7	49.3‡	64.6	6.3‡	13.2	.50‡	1.14
$2 \times \sqrt{2} \times$ S.E.	2.4		4.1		5.9		1.7		.15	
"F" Inoculations	111.13		52.35		85.33		285.66		274.81	
"F" Intervals	7.11		11.69		11.82		18.30		19.99	
"F" Replicates	2.07		.98		.55		.36		.65	

The 5% points for inoculations, intervals, and replicates are 3.98, 2.23, and 2.35, respectively; and the 1% points are 7.01, 3.07, and 3.29, respectively.

* *In terms of plant growth.*

† *Average weight of five plants.*

‡ *Indicates significant values between inoculated and control series.*

Discussion

These studies have indicated that a satisfactory method of securing infection of wheat seedlings with *H. sativum* or *F. culmorum* is to germinate the seedlings, and then, before they are transplanted, to immerse their roots for about seven to ten days in a nutrient solution in which the pathogen has been cultured. By delaying contact with the inoculum, the roots get a fair opportunity to develop; by decreasing or increasing the duration of the contact, the severity of the attack in the seedling stage may be more or less controlled at will. This technique permits good development of disease in a nutrient solution, and the method appears to possess advantages, should the plants be transplanted to a soil or sand substrate instead of to a nutrient solution. However, this possibility has not yet been demonstrated by experiment. Another advantage is that one may choose uniformly infected seedlings for study, which is important. (Experiment VI.)

Some infection and fairly satisfactory subsequent development of the disease were secured by adding a spore suspension of the pathogen and sugar directly to the nutrient solution, a few days prior to transplanting the seedlings. However, this method does not permit of the same degree of control or as much accuracy as is possible with the previous method. (Experiment V.)

On the other hand, when the spore suspension was added at the same time that the seedlings were transplanted, practically no disease developed. The addition of sugar to the solution made no appreciable difference in the results obtained. (Experiments II and III.)

Rather poor and uneven infection was secured when the seed was soaked in a spore suspension of *H. sativum* prior to germinating the seed. There

appears little to recommend this method in connection with the study of the disease in nutrient solutions. (Experiment IV.)

Of all the methods studied, the injection of spores of the pathogen into the crown tissue of the seedlings was least successful in producing infection. (Experiment I.)

Although a study of the relation of the composition of the nutrient solution to the development of the disease was not the primary purpose of the present paper, the evidence obtained from the above experiments indicated that this angle of the problem should be studied in detail. This is being done and the results will be reported later. Suffice it to say now that the plants were larger and more vigorous in certain nutrient solutions than they were in others. For example, in Experiments I, II and III, where no disease developed, the plants were less vigorous in the solutions having a high concentration of calcium, nitrogen, or potassium than they were in those with a low concentration of these minerals. With one exception, namely, the solution with a high potassium concentration (Experiment III), the plants were less vigorous in the complete nutrient solution. Apparently satisfactory development of disease occurred in the complete nutrient solution. (Experiment VI.)

A point of particular interest was that, in those experiments where very little or no disease developed, growth was better and chlorosis was less in certain solutions to which the pathogen was added as a spore suspension than in the controls. This occurred in all infested solutions of Experiments II and III. Evidently normal chlorophyll production in the plants was maintained in the presence of *H. sativum* or of *F. culmorum*, but not in the absence of either pathogen, despite the fact that ferric tartrate was added once per week. A tentative explanation is that these pathogens either increase the availability of iron in the solution or favor its assimilation by the plant. The importance of further study of this phenomenon, and its relation to the development of disease in culture solutions, is emphasized. The results of the experiments on this phase, now in progress, will be reported later.

Acknowledgment

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EFFECT OF PRECOOLING AND RATE OF FREEZING ON THE QUALITY OF DRESSED POULTRY[1]

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Abstract

The rate at which poultry is frozen has been shown to have no effect on the number of bacteria present, and little, if any, effect on the extent of surface desiccation or development of visceral taint. The development of taint appears to depend primarily on the period during which the product is held at temperatures above the freezing point, and little advantage is gained by freezing promptly after slaughter, since taint development occurs during thawing.

A quantitative study of the amount of fluid exuded (drip) after freezing and thawing whole birds shows that, regardless of the rate of freezing, the whole bird does not drip. Freezing does change the condition of the water in the muscle, however, since drip can be obtained from minced meat after freezing. If minced meat is frozen within 3 hours of slaughter, the amount of drip is somewhat variable but apparently independent of the rate of freezing. If the birds are stored for 24 hours or more at 0° C., prior to freezing, the typical curved relation between the amount of drip and the freezing rate is obtained, the drip decreasing as the freezing rate increases.

Using a constant rate of freezing (2.5 hours to pass from 0° to −5° C.), the amount of drip decreases as the storage time prior to freezing is increased. During storage at 0° C., the greatest decrease occurs during the first day, but continues for periods up to 2 weeks. At 10° C., little decrease occurs during the first 5 days, after which it decreases slowly until the product spoils. The amount of drip obtained at a given rate of freezing appears to be proportional to the amount of fluid obtained from the unfrozen material, showing that the drip is determined by the condition of the water in the original minced muscle. There were some indications that the state of the water in the tissue was partly determined by the pH, but the results were not conclusive.

Introduction

This investigation was undertaken to determine the effect of the rate of freezing on the quality of dressed poultry to be stored in the frozen state. When these experiments were under way it was found that the effect of the rate of freezing was conditioned by the treatment which the product received before freezing. The scope of the study was therefore enlarged to include the effect of precooling and storage prior to freezing.

It has been shown (2, 19, 20) that when fish and beef are frozen slowly in air, they exude a certain amount of fluid or "drip" when thawed. In addition to the quantitative losses, the eating quality also suffers, presumably due to the loss of certain of the flavor constituents (19, 21). The cause of drip is

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generally attributed to the formation of large ice crystals during slow-freezing (14, 17, 20), which exert a mechanical force (4) that may rupture the cells (11, 19) or prevent the cell constituents from reabsorbing the moisture (21), either because the rate of thawing exceeds the rate of diffusion back into the cells (19), or because the proteins are denatured by the processes involved in slow-freezing (5, 12, 16). Whether drip results from only one or a combination of these possible effects is not known, but it has been found (19, 20) that rapid freezing reduces both the size of the ice crystals and the amount of drip obtained when the product is thawed.

By analogy, it would appear that all meats should drip when thawed after slow-freezing. This does not appear to be the case, since slowly frozen pork and mutton (2, 19) evidently do not drip to any extent when thawed. The claimed superiority of quick-frozen poultry (10) appears to be based on the assumption that it is affected by freezing in a manner similar to beef and fish. On the other hand, it appears to be doubtful whether slowly frozen poultry does drip (10), but no direct evidence is available. Other investigations (13, 18) indicate that slow-freezing does not affect the eating quality. Further advantages have been claimed for the quick-freezing of poultry. These include: a reduction in the number of bacteria (7); a reduced development of visceral taint (10); and superior bloom and appearance, since the rate of surface desiccation is reduced (10, 22).

Most of these claims are based on the results of practice rather than experiment, and although their validity is not questioned, it seems probable that the commercial operator interprets "quick-freezing" in terms of a whole series of processes, rather than as the strict rate of freezing which the term implies. For instance, where quick-freezing is practised, the period between slaughter and freezing may be reduced, the birds eviscerated, and the entire marketing and distributing chain improved. The over-all process may therefore result in a superior product which cannot be attributed entirely to the rapid rate of freezing.

The major part of this investigation was devoted to the question of drip in frozen poultry. The effect of the rate of freezing on bacterial count, visceral taint, and surface desiccation was investigated in a preliminary way, and the results of the tests will be reported first.

Number of Bacteria

Haines (6) has shown that the temperature of freezing between $-5°$ and $-70°$ C., *i.e.*, rate, has little effect on the ratio of viable organisms before and after freezing. On the other hand, Heitz and Swenson (7) report that the number of bacteria on slow-frozen ducks is 1000 times greater than that on the quick-frozen product. Although this difference is attributed to quick-freezing, it appears that the two lots of ducks used were not subjected to exactly the same treatment, and the observed differences may therefore be due to other factors than the rate of freezing.

In order to provide material for these tests, chickens were stored for 3 weeks at 0° C. (32° F.). Since bacterial development is greatest on the surface, the meat was removed, minced, mixed, and allowed to stand overnight at room temperature. It was then placed in small metal containers, frozen at various rates, and stored for 2 days at −40° C. (−40° F.). The samples were thawed by placing them in air at 15° C. (60° F.) for 4 hours. They were then ground with sterile sand and extracted with sterile water. Counts were made after plating appropriate dilutions on beef-extract agar and incubating for 48 hours at 25° C. A second experiment was conducted in a similar manner except that the chicken meat was transferred directly from the room at 0° C. (32° F.) to the freezing chambers, and ground in the frozen condition.

TABLE I

EFFECT OF RATE OF FREEZING ON NUMBER OF BACTERIA IN MINCED CHICKEN MEAT

Rate of freezing (0° C. to −5° C.), hr.	Bacterial count per gm. of meat, $\log_{10}$	
	Exp. 1	Exp. 2
Check (unfrozen)	8.60	9.70
0.5	8.84	9.48
4.0	8.46	9.65
24.0	8.62	9.56

The results obtained are given in Table I, from which it is evident that the rate of freezing has little, if any, effect on the number of bacteria present. This finding appears to be in agreement with Haines (6). In practice, the quick-frozen product is usually frozen shortly after slaughter, whereas the slow-frozen product is frequently stored at a temperature near the freezing point for several days before freezing. Since bacterial growth at 32° F. appears to be relatively slow (8) it is difficult to account for the results of Heitz and Swenson (7), even on the basis of somewhat delayed freezing of the slow-frozen product. It appears that their material must have been exposed to temperatures above the freezing point for a considerable period, or else the material was not comparable in other ways. It is concluded from the results of the present investigation that quick-freezing confers no advantage over slow-freezing, from the standpoint of the bacterial numbers in properly handled poultry.

Surface Desiccation

Tests of the effect of the rate of freezing on surface desiccation were restricted to a study of the loss of moisture during freezing, and the effect of various other storage conditions will be described in another paper (1). The initial attempt to obtain quantitative information on this subject was made by determining the moisture content of the skin before and after freezing. This failed, owing to the marked variation in the moisture content of chicken skin, and the difficulty of obtaining a representative sample. The method finally adopted was to weigh the bird to an accuracy of 0.5 gm. before and after freezing. This gives the over-all shrinkage or loss during the freezing process, and possibly exaggerates the amount of moisture lost by the skin only.

Six birds, having an average weight of 6 lb., were precooled to 0° C. (32° F.). Three of these were frozen by hanging in a room at −40° C. (−40° F.), and three by hanging in a room at −13° C. (+7° F.). The time required for the centre of the birds to reach a temperature of 20° F. was 1.5 and 16 hours respectively. The freezing process resulted in a loss of 3, 2, and 2 gm. for the quick-, and 4, 3, and 3 gm. for the slow-frozen birds. This corresponds to a mean over-all loss of 0.08% and 0.12% respectively, or a difference of 0.04% between the two rates of freezing. Even if this loss came entirely from the skin, it could hardly be expected to render the product at all independent of the storage conditions under which it is subsequently kept, since these will be shown (1) to have a marked effect on the rate of development of surface marking. In commercial practice the birds would be frozen after packaging, and although this would reduce the rate of freezing, it would also reduce the shrinkage to less than the values reported above.

Development of Visceral Taint

Mandeville (10) states that one of the main advantages of quick-freezing is to prevent the development of off-flavors which take place after death; but experimental evidence to support this statement is lacking. Commercially, quick-freezing is frequently practised on eviscerated poultry, and the benefits of these two distinct processes, evisceration and quick-freezing, may have become confused. Nevertheless, it is possible that the rate of freezing may affect the development or transmission of taint from the viscera or other regions.

There are no adequate methods for measuring the amount of taint given off by a particular organ or region during freezing, or its effect on the odor or flavor of the meat after transmission. In these tests the whole viscera were used and an attempt made to determine: the effect of prompt *versus* delayed freezing; the effect of both freezing and thawing separately and combined; and the effect of storage at 0° C. (32° F.) without freezing. The method used was based on the "enfleurage" method (15), or the absorption of the odor by a fatty substance. The general procedure consisted of removing the viscera, wrapping them in blotting paper supported by a cotton bag, and placing the whole in quart sealers previously coated thinly on the inner surface with Crisco. The sealers were stoppered and treated in accordance with the requirements of a particular experiment. Quick-freezing was accomplished by immersing the sealer in an ethylene glycol water bath at −33° C. (−28° F.), and slow-freezing by insulating the sealer with about one inch of cotton wool and placing in air at −13° C. (7° F.). The exact time required for freezing under these conditions was not determined. Thawing was accomplished in all cases by placing the sealers in a cabinet at 10° C. (50° F.) for two days. After thawing, the viscera were removed, and the sealer was stoppered tightly and left at room temperature for one day. Five persons then estimated the intensity of the odor in accordance with the following score card: 0—no foreign odor distinguishable; 1—ethereal but not distasteful; 2—unpleasant;

and 3—intense and disagreeable. The values reported in Table II are the average of the five scores made in duplicate.

The summarized results given in Table II show some irregularity, which is to be expected in subjective tests of this sort, but they are reasonably consistent. It appears that tainting substances do develop in the viscera during slow-freezing (Exp. I), thawing (Exp. II), precooling prior to freezing (Exp. III), and storage in the unfrozen state (Exp. IV). The advantage of quick-freezing the warm viscera, observed in the first test, is not shown by the second. The elapsed time before freezing appears to be more important than the rate of freezing. The taint apparently develops largely during the thawing process. No evidence of free drip was obtained at any rate of freezing. These results indicate that visceral taint is entirely a question of the period during which the product is held above the freezing point. Evisceration rather than prompt or quick-freezing appears to be the obvious solution for this difficulty.

TABLE II

DEVELOPMENT OF VISCERAL TAINT

Exp. No.	Treatment of viscera	Processes involved in test	Intensity of odor* (average score of 5 persons)	
			Slow-frozen	Quick-frozen
1	Not precooled prior to freezing	Freezing, 2 days' storage at −28° C., and thawing.	2.4	0.7
2	Not precooled prior to freezing	Freezing only.	0.6	0.4
		Thawing only.	2.6	1.4
3	Precooled 24 hr. before removal from bird	Freezing, 2 days' storage at −28° C., and thawing.	1.7	1.4
4	Not precooled prior to placing in jar	Not frozen, stored for 6 days at 0° C.	2.8	

* *Maximum intensity score—3.0.*

It is difficult to interpret the taint intensities observed in terms of flavor of the meat. Poultry stored at 0° C. (32° F.) for six days, or even longer, is generally considered of good eating quality, but this treatment resulted in the most intense odor recorded. Possibly, more taint would be developed by the viscera during storage in the frozen state, a condition not included in these studies. Since the amount of taint developed during frozen storage would be more likely to depend on the temperature and other storage conditions than on the rate of freezing, this fact would appear to furnish a further reason for evisceration.

Exudation of Tissue Fluids (Drip)

METHOD

It is convenient at this point to present the method employed for determining the amount of fluid exuded after freezing, *i.e.*, drip. Essentially, it

consisted of a slight modification of that used by other investigators (2, 17) for determining the drip in beef and fish. Other methods were also studied for purposes of comparison and will be discussed later in relation to the results obtained.

A sufficient quantity of meat for the experiment in question was obtained from several chickens, ground, and thoroughly mixed. Samples of about 100 gm. were taken, placed in tared metal dishes, about 3 in. in diameter and 1 in. deep, having removable tops and bottoms. The samples were weighed accurately, frozen at the desired rate, stored at the freezing temperature for at least 2 days, and then thawed by placing them in a tight-fitting cylinder, jacketed with water at 15° C. Thawing by this technique required about 4 hours. The drip was then determined by replacing the tops and bottoms of the cans with several layers of blotting paper, the layers being held in place by a weight equivalent to 1 gm. per sq. in., and allowing them to stand for 20 hours, at 0° C. The blotters were removed, the sample reweighed and the loss computed. The loss in weight of an unfrozen control sample was similarly determined and subtracted from that of the frozen sample to obtain the quantity reported as net drip. Usually triplicate, and frequently quadruplicate samples were used for each test and control measurement.

In order to obtain an estimate of the accuracy of this method, the standard error of the mean of triplicate samples was computed for 20 determinations chosen at random. It was found to be $\pm 0.53\%$. Similar calculations for the controls, which had a lower total loss of weight, gave an error of $\pm 0.20\%$. The standard error of the difference, *i.e.*, net drip, would therefore be $\pm 0.57\%$, and the 5% point approximately 1.1%. Differences in net drip greater than 1.1% can therefore be considered significant.

Drip in Relation to Rate of Freezing

To determine the amount of drip obtained at different rates of freezing, preliminary experiments were made on: eviscerated whole chickens; skinned half chickens; meat cut into approximately 1-in. cubes; minced dark meat; and minced light meat. The birds frozen whole were eviscerated to avoid the possible accumulation of drip in the body cavity, and the skin was removed from the half-birds used in the second experiment to prevent it from retaining any drip. The method employed in these instances was to place the birds in moisture-tight cans, freeze them at the desired rate, thaw at 10° C. for 2 days in a manner which permitted any liquid to accumulate in the bottom of the can, and then measure the amount of liquid. This procedure gives an estimate of the free drip plus any liquid that may have evaporated from the birds and condensed on the can during the freezing process. The cut and minced meat samples were tested by the method already described.

The results obtained are reported in Table III. It is evident that the amount of drip obtained from whole birds is negligible regardless of the rate of freezing. Removal of the skin, cutting, and mincing the meat progressively

TABLE III

EFFECT OF RATE OF FREEZING ON WHOLE BIRDS, AND CUT AND MINCED POULTRY MEAT

Material		Freezing rate: time to pass from 0° C. to −5° C. (32° F. to 21° F.)		
		1 hr.	8 hr.	18 hr.
Whole birds (eviscerated)*	Drip %	0.05	—	0.15
Half birds (skinned)*	Drip %	1.0	—	1.9
Cut meat, (1-cm. cubes approx.)†	Drip %	1.4	2.3	2.5
Dark meat (minced)†	Drip %	2.0	6.5	6.8
Light meat (minced)†	Drip %	2.9	7.4	8.7

* *Drip estimated from weight of free liquid obtained.*

† *Drip estimated from differential weight loss of frozen and unfrozen samples after absorption of liquid with blotting paper.*

increase the amount of drip obtained, and on the minced tissue the effect of different freezing rates can easily be ascertained. Although all the methods indicate that the amount of drip decreases as the freezing rate increases, the observed differences in the amount of free drip obtained from the whole, and half chickens could be attributed to experimental error. The observed differences, using cut meat and absorption with blotting paper, are too small to establish any definite relation between drip and freezing rate, so that minced meat was used for subsequent measurements. The results in Table III show that light meat yields more drip than dark meat, but as the difference was not great, both kinds were mixed and ground together in preparing material for later work.

Before initiating the main series of investigations, a number of chickens of different weight (age) from different sources were studied. All of these yielded essentially the same amount of drip at the same freezing rate. It was found, however, that the period allowed for precooling between slaughter and freezing did affect the quantity of drip obtained. In consequence, two post-slaughter treatments were used; in one, the meat was minced and placed in the freezer within 3 hours of slaughter, and in the other, the birds were pre-cooled for 24 hours at 0 °C. (32° F.) before being cut up, minced and frozen. Unless otherwise stated, the birds were all starved for 24 hours prior to slaughter.

The effect of various rates of freezing on drip formation is given in Fig. 1 for minced meat frozen within 3 hours of slaughter, and for minced and cut meat after 24 hours precooling. The rate of freezing is expressed as the time required for the product to pass from 0° C. (32° F.) to −5° C. (21° F.). As indicated by the results given in Table III, cut meat yields less drip than comparable minced samples. The curves from the cut and minced meat, however, have the same general form. After precooling the birds for 24 hours, the quantity of drip obtained from minced meat decreases with the rate of freezing. The quantity of drip increases quite rapidly up to about a 4-hour freezing period, after which it increases relatively slowly so that longer freezing

times give about the same amount of drip. This is the typical relation which other investigators (11, 17) have found with beef and fish. It is evident from the curve that a freezing time of about an hour is required if the quantity of drip is to be reduced to half that obtained by slow-freezing.

The amount of drip obtained from minced chicken meat frozen within three hours of slaughter was somewhat variable, but was always equal to, or greater than, the maximum quantity obtained from birds that had hung overnight (Fig. 1). The fact that large quantities of drip were obtained from quick-frozen material of this sort indicates that some post-mortem change takes place during precooling, which tends to reduce the quantity of drip obtained. The relatively constant quantity of drip obtained at all freezing rates may, therefore, be the result of two opposing tendencies, slow-freezing tending to increase drip, but allowing the post-mortem changes which reduce it to take place and *vice versa*.

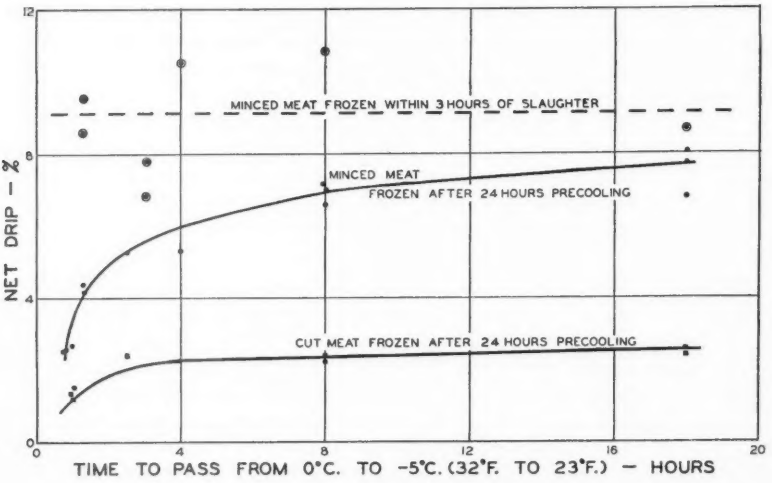

FIG. 1. *Drip in relation to rate of freezing.*

It should be noted that although the drip from minced meat is relatively high and is related to the rate of freezing, whole chickens show negligible drip at any freezing rate. This fact brings up the question as to the nature of the deterioration, if any, that occurs when chicken meat is frozen. In other words, does the change in the water relations caused by freezing, as evidenced by the drip in the minced meat experiments, cause deterioration, or must this drip escape from the tissues before any effect is noted? If the latter view is accepted, then the rate of freezing has no effect on the quality of poultry since no fluids escape from the whole carcass.

Drip in Relation to Storage before Freezing

Further investigations were undertaken to determine the effect on drip of storage prior to freezing, since investigations (4) on beef indicate that the period between slaughter and commencement of freezing had no definite effect on the quantity of drip obtained. One rate of freezing was used throughout, *i.e.*, 2.5 hours to pass from 0° C. to −5° C., the product being stored at 0° C. (32° F.) and 10° C. (50° F.) for various periods prior to freezing. The higher storage temperature was used to determine whether the observed changes occurred more rapidly as the temperature increased.

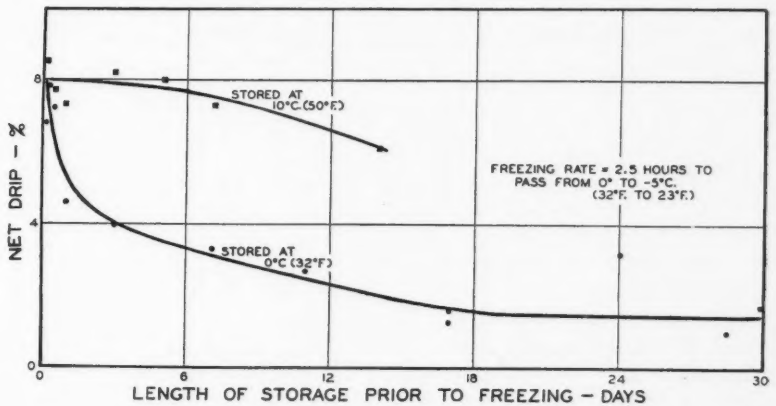

Fig. 2. *Drip in relation to storage before freezing.*

The results obtained were used to construct the curves in Fig. 2. It is evident that storage at 0° C. prior to freezing decreases markedly the amount of drip during the first 24 hours, after which there is a slow decrease during 18 days' storage. Beyond that time the drip is more variable, probably due to incipient putrefaction, but appears to remain reasonably constant throughout the storage of the product at this temperature. This change in the quantity of drip obtained during storage cannot be attributed to evaporation since all birds were stored in closed containers at 100% relative humidity. The birds stored at 10° C. (50° F.) showed a constant drip of about 8% for the first 6 days after which it fell off slightly until the 14th day when the test was terminated owing to putrefaction of the meat.

These experiments show that certain rigor and post-rigor changes occur during storage at 0° C. which tend to reduce the drip obtained after freezing. These changes do not take place to any extent at 10° C. It is possible that two types of change can take place: one during rigor, which causes a rapid increase in the water-retaining capacity of the tissue; and another which acts in the same direction but more slowly, as indicated in the lower curve. If this explanation is correct, the difference between the curves obtained at

0° C. and 10° C. is that the first change does not occur at the higher temperature.

These results, which are typical of many more experiments, show that the amount of drip obtained depends on the initial condition of the tissues as well as on the rate of freezing. Further evidence favoring this view arose from the observation that when the drip in the unfrozen control was high, the total drip in the frozen sample at a given rate of freezing was also high, and *vice versa*. The difference between these two quantities, or the net drip, usually showed the same behavior. In order to confirm this observation, the percentage of total drip obtained from a number of frozen samples was plotted against the drip obtained from the corresponding unfrozen controls. The resulting graph is shown in Fig. 3. It is evident that the amount of drip

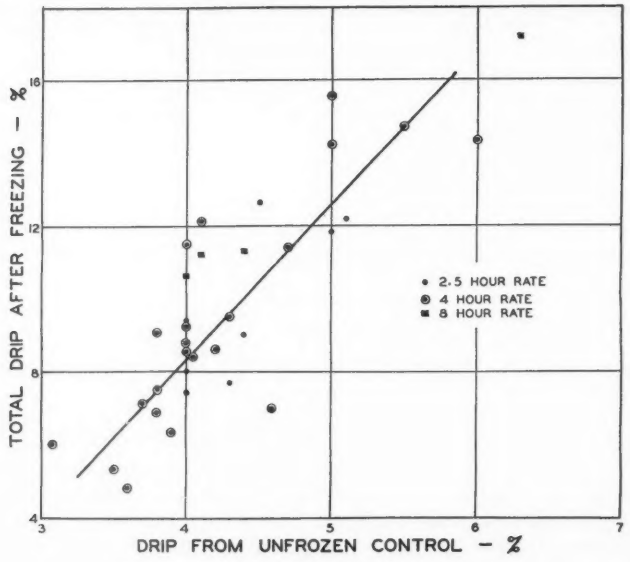

FIG. 3. *Drip in relation to condition of muscle.*

obtained after freezing depends on the tenacity with which it is held in the original unfrozen sample. Storage prior to freezing causes the tissues to retain their moisture more firmly, but other factors may also affect the condition, and the nature of the changes that take place is still obscure.

DRIP IN RELATION TO CONDITION OF MUSCLE TISSUE

In an attempt to determine the nature of the conditions governing the state of water in muscle tissue, and consequently the amount of drip, the first experiment undertaken was an analysis of the drip. The drip was obtained from approximately 500-gm. samples of meat, by subjecting the thawed

samples, contained in narrow mouthed percolators, to a pressure of 0.35 lb. per sq. in. for 3 days at 0° C. (32° F.). A slight suction was applied from time to time to remove the free liquid which collected in the voids. Samples of chicken meat were obtained immediately after slaughter, and also from birds stored 24 hours before mincing and freezing. Both tests included an unfrozen control and samples frozen at two rates. The quantity of drip thus obtained provided material for analyses and also established that there was a marked relation between the amount of free drip from minced tissue and the quantity obtained by the blotting paper method. After three days, when the last of the drip had been removed, 500 ml. of 2% KCl was added to the percolators, allowed to stand for 24 hr., and then drained off for analysis.

The results of the analysis performed on the exuded fluid and percolate from each sample are reported in Table IV. It is evident, in spite of the fact that the amount of drip obtained from the different samples varied in accordance with the treatment and freezing rate, that the composition of these fluids, with respect to the analyses performed, was essentially the same for the material frozen immediately or after 24 hours, and for the unfrozen or frozen samples. It appears, therefore, that the fluid exuded from the tissue is of relatively constant composition regardless of the treatment, or the amount obtained. This finding is in agreement with that obtained by Empey (4) on beef.

TABLE IV

COMPOSITION OF DRIP

Rate of freezing (time to to pass from 0° C. to −5° C.), hr.	Free drip, %	Original drip					Percolates	
		pH	Total solids, %	Non-combustible solids, %	Protein nitrogen, %	Non-protein nitrogen, %	Total nitrogen, %	Organic matter, %
Frozen within 3 hr. of slaughter								
Unfrozen control	5.7	6.15	14.8	1.20	1.68	0.48	0.68	5.0
1.3	14.3	6.20	15.0	1.24	1.60	0.48	0.68	4.8
18.0	14.4	6.05	14.7	1.23	1.76	0.48	0.66	5.3
Stored for 24 hr. at 0° C. prior to freezing								
Unfrozen control	4.2	6.35	14.4	1.26	1.70	0.44	0.64	5.2
1.3	8.4	6.30	14.8	1.23	1.72	0.43	0.63	4.7
18.0	12.0	6.25	14.6	1.19	1.70	0.46	0.61	5.6

Empey found that the period between slaughter and commencement of freezing has no effect on the quantity of drip from beef. Furthermore, he states that the amount of drip is minimal in muscle tissue at about pH 6.3 and that conditions more acid than this tend to increase the quantities obtained. These results appear to be conflicting, since the formation of lactic acid in the

muscle after slaughter usually results in a gradual decrease in pH which attains finally a value considerably more acid than pH 6.3. If pH is the principal factor determining the loss of fluids, then it would appear that the drip should increase for a certain period after slaughter. Chicken meat may not be comparable with beef, but the fact that the drip definitely decreased with time from slaughter, and the apparent conflict in Empey's results, rather indicate that another factor than the pH of the tissue is involved.

TABLE V

EFFECT OF STORAGE PERIOD BEFORE FREEZING, AND pH, ON DRIP
Rate of freezing—4 hr. to pass from 0° to 5° C.

Test No.	Pre-slaughter treatment	Storage period at 0° C. (32° F.) prior to freezing, hr.	pH		Drip		
			When minced	When thawed	Unfrozen control, %	Total (frozen sample), %	Net %
1	Normal: birds pre-starved	4	5.8	—	—	15.6	—
2	24 hr. before slaughter	24	6.0	—	—	9.3	—
3		144	6.1	—	—	8.4	—
4	Birds pre-starved 24 hr.	4	6.0	—	—	11.9	—
5	received injections of insulin prior to slaughter	24	5.8	—	—	5.2	—
6		144	6.0	—	—	4.3	—
			(Uncut meat)				
7	Normal: birds pre-starved	0.5	7.0	6.3	6.0	14.3	8.3
8	24 hr. before slaughter	24.0	5.5	5.9	—	9.0	—
9	Birds pre-starved 24 hr.	0.5	7.2	6.6	5.0	14.4	9.4
10	received injections of sodium iodoacetate prior to slaughter	24.0	5.6	6.1	4.3	9.5	5.2
11	Normal: birds pre-starved	24	—	5.9	4.1	12.1	8.0
12	24 hr. before slaughter	120	—	5.8	3.7	7.0	3.3
13	Pre-starved for 24 hr. and	24	—	6.2	3.8	6.8	3.0
14	exercised prior to slaughter	120	—	6.1	4.6	7.2	2.6
15	Fed until slaughter	14	—	5.9	3.8	9.1	5.3
16		120	—	5.9	3.9	6.4	2.5
17	Birds received injections of	24	—	5.8	4.7	11.4	6.7
18	both insulin and glucose prior to slaughter	120	—	5.7	3.8	7.5	3.7

These considerations led to some experiments in which the pH as well as the drip was studied, after various storage periods. Preliminary measurements on the pH of minced chicken muscle indicated that it usually fell within the narrow range of 5.8 to 6.1. Attempts were therefore made to extend the range of pH values obtained. Three experiments of this sort were made.

The first two were concerned primarily with the changes in pH and drip within 24 hours after slaughter, and the third with the changes that occur after a 24-hour storage period. The initial experiment included normal, pre-starved birds, and similar birds that received 120 units of insulin (3) prior to slaughter. A comparison of the changes that occur in normal, pre-starved birds, with those that take place in similar birds injected with 0.04 gm. of sodium iodoacetate (9) prior to slaughter was made in the second experiment. The final test included the following treatments: normal, pre-starved birds; birds similarly starved but vigorously exercised for 10 min. before slaughter; birds fed until slaughter; and birds that received both intravenous glucose and subcutaneous insulin 90 min. before slaughter. All measurements on this material were made 24 hours or more after death.

The results of the experiments are given in Tables V and VI. All the pH measurements were made with a glass electrode and the values are corrected to 20° C. (5), although the majority of the observations were made at lower temperatures. Owing to the limited amount of material available for the first experiment, only the total drip from the frozen sample was tested. The results show that the pH of tissue from both the normal and insulin-treated birds was quite similar at the time of mincing, and changed very little with time of storage. On the other hand, the quantity of drip from the insulin-treated birds decreased more rapidly during storage than did that from the normal birds. This suggests a relation between carbohydrate metabolism and drip, although the pH values show no evidence of any great difference in the amount of acid produced. In the second experiment, it was found that the injection of iodoacetic acid only maintained the neutral condition for a matter of 2 hours (Table VI) or less, after which it behaved as the normal sample. Therefore, no conclusion is possible from this test, since the pH and drip values were the same from both normal and treated birds.

The final experiment involved a number of treatments, and the drip and pH (when thawed) measurements were made one and five days after slaughter. In all cases, the drip decreased during storage and the tissue tended to become slightly more acid. The pre-starved and exercised birds were the most alkaline throughout and showed the least drip. This result appears to support Empey's findings. The pH and drip for the other treatments were essentially the same throughout.

TABLE VI

CHANGES IN PH OF CHICKEN MUSCLE DURING STORAGE AT 0° C.

Time from slaughter, hr.	pH of whole muscle	
	Normal birds	Birds injected with sodium iodoacetate
0.5	7.0	7.2
1.5	6.5	7.3
4.0	6.2	6.3
6 0	5.6	5.8
24.0	5.5	5.6

Investigations relating to pH and drip formation in chicken meat are complicated by the fact that no evidence of free drip from the whole carcass was obtained. This,

together with the possible variable behavior of the muscle from different chickens, led to a study of drip in relation to the effect of pH and storage on beef, pork, and mutton. The results of these investigations will be reported shortly.

It is of interest to report the observation that the pH appears to affect the color of the muscle. In these experiments, the pH range obtained in samples 24 hours after slaughter was relatively narrow, yet the more acid samples had a light pink color while the more alkaline samples had a darker appearance, tending toward a brown. This observation may be of some significance and is being studied in a more quantitative manner on other meats.

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THE INFLUENCE OF NUTRITIONAL AND CLIMATIC FACTORS ON WOOL GROWTH AND QUALITY

I. STATEMENT OF PROBLEM AND EXPERIMENTAL PROCEDURE[1]

By J. P. Sackville[2] and J. E. Bowstead[3]

Abstract

A five-year study has been conducted on the effect of humidity, temperature, date of shearing, plane of nutrition, and protein or mineral intake, on wool growth and quality. The results indicate that significant differences in wool growth and quality may be obtained by varying the shearing dates and the plane of nutrition of the ewes. Little or no effect was evident when ewes were subjected to differences in temperature or humidity, or in mineral and protein intake levels.

Following a conference in the City of Toronto in May, 1927, an Associate Committee on Wool was organized by the National Research Council of Canada, in co-operation with Canadian producers and manufacturers of wool, government departments, and agricultural colleges. It was realized that Canada depended too largely on wool from other countries and it was felt that if Canadian wool could be improved sufficiently to increase its use in domestic manufacture, it would be beneficial to all phases of the industry.

A sub-committee was formed to consider what studies might be undertaken in the field of production. As a result of a survey of certain sheep ranches in western Canada, it became evident that the greatest hope for the improvement of Canadian wool lay in breeding experiments. It was also obvious that in such experiments attention should be paid to mutton quality as well as to wool.

Based on a report of the sub-committee, two experimental breeding projects were established, one at the University of Saskatchewan, Saskatoon, and the other at Cardston, Alberta. Both were assisted financially by the National Research Council, and the experiment at Cardston was supervised by a small group of the Associate Committee on Wool.

In order to evaluate a possible improvement in wool through breeding, it was considered necessary to investigate any effect attributable to general environmental conditions. At that time little information was available concerning the relation of climate and nutrition to wool growth and quality. It was arranged that an investigation of this particular problem, under controlled conditions, should be undertaken at the University of Alberta. Studies were planned to demonstrate the effect on wool growth and quality of (i) humidity, (ii) temperature, (iii) early and late shearing, (iv) plane of nutrition, (v) protein levels, and (vi) mineral intake. Wool samples from the different groups of sheep were forwarded at regular intervals to the National Research Laboratories in Ottawa for critical analysis. For several years, entire fleeces from

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all the lots were subjected to special grading at the warehouse of the Canadian Co-operative Wool Growers, Weston, Ontario.

The investigation covered a five-year period, 1929–30 to 1933–34, and was supported financially by the National Research Council. The results, obtained at the University of Alberta, in the National Research Laboratories, and at the Weston warehouse of the Canadian Co-operative Wool Growers, will be presented in a series of three papers, of which this is the first.

Review of Literature

One of the first published statements concerning the relation of wool growth and quality to the animal's environment was reported by Youatt (39), who stated that "about 1776 a Dr. Anderson noticed that fibre diameters were greater when grown under high temperatures." In 1806, Dr. Parry reported in the Bath Society papers, that he had observed a "sort of gross connexion between food and the quality of the fleece," but was not certain "that coarseness of wool and tendency to grow fat are connected." Further evidence of how climate and nutrition influenced the gross weight and wool quality was reported by numerous individuals, based on observations and crude experimentation. It was generally observed that a variation in wool occurred in different regions and in the same region from year to year.

Five or six German investigators have studied seasonal wool growth and have shown that practically none occurred during the last two months of the yearly production, while approximately two-thirds took place during the first six months after shearing. In studies on the monthly wool growth of Rambouillet (6), Hampshire, and Corriedale (7) ewes, Burns found that the seasonal variations were not as great as those reported by the German investigators. These three breeds produced, during the first six months after shearing, 54, 52 and 57% respectively of their yearly growth. Lush and Jones (23) report that the effect on the fleeces of very droughty seasons is greater than the influence of age. Burns (6) states that Nordmeyer secured more wool growth during those months when green feed was available, and less when the ewes were suckling lambs.

The difference from year to year at the University of Alberta, in total raw wool produced by a flock numbering about 225, has been over 300 pounds. This indicated that after prolonged winters of low temperatures the raw fleece weights were less than those clipped after moderate winter conditions.

Hardy and Tennyson (21) found that the rate of wool growth and the fineness varied throughout the year, both growth and coarseness being greatest in summer and fall, and least in mid-winter. They also found that wool production is associated with general thrift and condition of the sheep as indicated by their weight. The period of least growth occurred in ewes usually during lambing time and the 45 preceding days. Numerous experimenters have shown that wool growth and quality are affected by differences in feed supply which may be the result of natural feeding conditions or controlled feeding trials. Duerden (13) found that wool grew faster during the

summer, when the sheep were in pasture, than during the winter months. Studies by Tinley (35) indicated that thick, lush herbage tends to stimulate the production of a larger and coarser grade of wool than does the thin, sparse herbage associated with the finer grades. Smith and Hussain (32) found that growth of wool was affected by the lambing season and by periods of abundance or lack of feed. On the other hand, Fraser (17) found that wool grew at a uniform rate throughout the year when sufficient pasture was provided.

In controlled feeding trials where wide differences in the nutrition of ewes could be secured, marked variations in growth and quality of wool have been noted. Wilson (38) compared the wool production of sheep on a fattening ration with that of a second group on a sub-maintenance ration, and found differences of 41% in fibre growth, 343% in grease weights, 319% in scoured weights, 207% in fibre strength, and 26.5% in fibre diameter, in favor of the fattening ration. He concludes that a "state of semi-starvation such as thousands . . . . . of sheep are subjected to on our western ranges during two to four months of the year . . . . . will not produce good wool." Weber (37), and Snell (34) secured similar results, in that sheep maintained on a low plane of nutrition produced shorter and finer wool, and much less scoured wool. Mitchell (27) fed a group of sheep on a sub-maintenance diet, and although the animals were in a negative energy and nitrogen balance for over 200 days, they stored energy and nitrogen in their wool at approximately normal rates.

One authority does not believe that wool growth can be easily affected by changes in level of feeding. Joseph (22), as a result of his experiments, concluded that "in sheep of fine-wool breeding, the organs which are concerned with the secretion of the wool fibre are not easily subject to such influences as changes in the level of feeding, especially for periods of 5 or 6 months, if the sheep remain in normal health; that the quality of the wool fibre is affected not at all, and that the quantity of fibre may be modified only slightly, as long as the sheep remains in normal health." He advocated that "level of winter feeding should be based on the needs of the sheep for health and condition for withstanding rigorous winter climatic conditions rather than for wool". Similarly Cooke (8) found that wool growth is only slightly reduced when the ration is insufficient for maintenance.

Dowell and Bowstead (12) conducted a series of pregnancy experiments and concluded that "any roughage or combination of feeds that maintains the ewes in good, vigorous condition tends to produce a heavier fleece than the less satisfactory feeds." There appears to be ample proof that the plane of nutrition does affect wool growth and quality, though there is much to be learned concerning the extent and limitations of such an effect.

Since the wool fibre is chiefly protein (keratin), research workers have done considerable work to determine the effect on wool growth of feeding low and high levels of protein, or special proteins, or minerals high in sulphur. As early as 1890, Cooke and Jones (9) in Vermont fed a carbohydrate ration to one group and a nitrogenous ration to another. Their results showed that

there was no measurable difference in diameter of fibre. They pointed out, however, that the wool fibres of a few sheep that "did not do well" were "shrunken in diameter". About the same year, Craig (10) at Wisconsin conducted a similar trial and concluded that sheep fed on the nitrogenous ration clipped 2.4 pounds more unwashed wool and 0.6 pounds more washed wool than those on the carbohydrate ration. The additional weight in the unwashed wool was credited in a limited degree to the amount of yolk present.

Marston (24), feeding blood meal to increase the protein level, found that the addition of the supplement increased the raw and clean fleece weights by over 35%.

Fraser (18) reported that at the Rowett Institute "it has been found that the feeding of a relative excess of protein gives a 20% increase in crude fleece weight. Conversely the feeding of a protein-deficient ration gives a 20% decrease in fleece weight, ... these figures are closely reflected in the scoured fleece yields." However, in collaboration with Roberts, Fraser (20) later reported an experiment in which soybean meal was used to increase the level of protein intake. They concluded that "increasing the protein in the ration caused no significant difference between the two groups in any of the wool characteristics measured," which were dry weight, fineness, fibre length and grease. A seemingly opposite effect was later obtained by Fraser, who with Nichols (19) secured an increase in wool growth by adding carbohydrates to what they termed a balanced ration.

The protein problem has been further studied in relation to the sulphur and cystine content of feeds. When Marston and Robertson (25) found that the cystine content of wool was constant, they assumed that the quantity of cystine available in the diet must be a factor limiting the quantity of wool that can be produced. Increased wool growth was secured by Marston (26) when cystine was administered to one sheep on a low protein diet, and the response was three times greater "when cystine was injected subcutaneously so as to avoid destruction of the free amino acid by bacteria in the alimentary canal." Barritt and King (2), and later, Rimington (29 and 30) found that the cystine in wool was not constant; they suggested that probably sheep had the ability to synthesize cystine. The work of Bosman (4) also indicated the synthesis of cystine. He found that the sulphur content of wool grown during periods of adequate nutrition is higher than during periods of scarcity, and is not related to the sulphur in the diet. In his experiments, controlled feeding of maize meal (poor in cystine) for a year failed to bring about marked decrease in either fleece weights or in sulphur content of the wool. It would appear that more cystine is used in producing wool than can be accounted for in the food eaten.

Barritt and King (3) likewise found that the variation in the cystine content of rabbit fur was due more to seasonal factors than to the quantity of cystine fed in the diet. Russell (31) found that when rations high or low in sulphur were fed no effect on the gross weight of the fleece, or on the percentage of clean wool, of sulphur in pure wool fibre, or of yolk, was apparent. Darlow *et al.* (11)

likewise failed to get significant responses from variations in amount of protein, or cystine, fed to wethers. Van Wyk *et al.* (36) obtained no increase in wool growth when a low protein ration was supplemented with either cystine, sulphates, potassium thiocyanate, or sulphur.

From the experiments reviewed above, one could conclude that the relation of dietary protein, cystine, or sulphur levels to wool growth has yet to be established. Further, the ability to produce normal wool probably is not lost during short periods of caloric or protein deficiencies when there is no impairment to health.

From our present knowledge of nutrition we believe that wool growth and quality may be indirectly affected by deficiencies of certain vitamins and minerals that are not associated with wool growth but with other bodily functions. Numerous experiments could be cited to show the need of various vitamins and minerals for the maintenance of growth, lactation, reproduction and health, but a survey of the literature has failed to reveal where wool growth was a factor in determining the role of any one vitamin or mineral. Pregnancy trials at the University of Alberta have proved that oat green feed is deficient in calcium. Numerous studies elsewhere have shown that there may be phosphorus or vitamin deficiencies in certain roughages. The prevalence of goitre has indicated deficiency of iodine. But in none of these trials was wool growth studied. However, there is reason to assume that unthriftiness caused by a mineral deficiency would be followed by decreased wool growth. Duerden *et al.* (14) secured a 32% decrease in fleece weight and a considerable decrease in body weight when they fed sheep on a phosphorus-deficient diet. Riggs (28) reported "improvement in growth, lustre and elasticity of the new wool grown after the administration of ferric ammonium citrate."

The purpose of summarizing the foregoing experiments has been to show how meagre and fragmentary is knowledge concerning the relation of wool growth and environment. It is generally accepted that a relation does exist. This is based on the differences in quantity and quality of wool produced by sheep maintained under different conditions of soil and climate over a long period of time. However, convincing evidence is lacking of the effect of a measurable difference in any one of the environmental factors. Some experimenters have attempted to measure such an effect. So far the results are either incomplete or contradictory.

To obtain the maximum growth and quality of wool, the environmental factors involved in its growth must be determined; then the sheep may be raised under optimum conditions. In many cases, the common methods of feeding and general management now in use are not conducive to maximum wool growth and quality. To shed more light on the problem of wool growth and to determine the influence of certain environmental factors on growth and quality of wool, the following trials were conducted.

Outline of Experiment

In all of the five trials, nine lots of ewes (eight in each) were used. Each group was placed under the environmental condition indicated in the following list:—

Lot 1. High temperature, high humidity, early shearing.
2. High temperature, low humidity, early shearing.
3. Low temperature, high humidity, early shearing.
4. Low temperature, high humidity, late shearing.
5. Low plane of nutrition.
6. Medium plane of nutrition.
7. High plane of nutrition.
8. Medium plane of nutrition, plus casein.
9. Medium plane of nutrition, plus minerals.

Lots 1 and 3 were the high and low temperature groups.
1 and 2 were the high and low humidity groups.
3 and 4 were the early and late sheared groups.
5, 6 and 7 were the low, medium, and high plane of nutrition groups.
8 and 6 were the high and low protein groups.
9 and 6 were the high and low mineral groups.

Significant differences in performance of the contrasted groups, therefore, would be a measure of the effect of the different environmental and nutritional conditions imposed.

Sheep Used and Method of Allotment

During the first three trials, grade and purebred ewes of different mutton breeds were used, while in the last two, ewes of Rambouillet breeding were used. This change was made to reduce the variability of the animals in any one lot.

In the first three trials (grade and purebred sheep), each comparable group had an equal number of ewes of each breed, as similar for age, weight, fleece weight, fleece length and quality as it was possible to obtain from the University flock of over two hundred. By this method of allotment it was assumed that there would be comparable pairs of individuai animals in the respective lots. It was also assumed that by repeating the trials, sufficient data would be obtained to show significant differences in the performance of comparable groups.

However, owing to the variability of the results within the lots and because the results of the first year differed from those of the second in certain instances, it was deemed advisable to use animals that were more uniform in breeding, age, and wool characters. Range ewe lambs and yearling ewes were purchased in the fall of 1932 for the 4th and 5th trials. These ewes were from the same flock and were the result of several generations of Rambouillet breeding on the sire's side of the pedigree.

Although in all trials the allotment was made to secure equality of comparable lots, there was considerable variation among the individuals of any one lot. According to the Committee on Methods of Investigation for the American Society of Animal Production (1), "uniformity between lots is a *sine qua non* of experimental work. Uniformity within lots is desirable in the investigation of most problems, but is less important than that the lots be uniform when compared with each other." Dunlop (15) strongly criticizes the use of groups showing variation within themselves, as well as the group-feeding method used. He states, "no basis of allotment, at present available, predicts the relative or quantitative growth rate of animals with any degree of certainty" and refers the reader to numerous trials for proof. Snedecor and Culbertson (33) proved in a trial with pigs that an experimenter can reduce the probable error of the resulting mean differences by estimating the animals' performance or "outcome" and allotting them uniformly on this basis.

However, it was assumed that the method of allotment used in these trials would reduce the error of the mean differences below that resulting from random grouping of the experimental animals.

Nutrition of Animals

Feeds commonly fed to sheep in Alberta were used throughout. The amounts of hay and grain (with the exception of Lots V and VII, the low and high plane of nutrition groups) were such as would carry the ewes through the trials in fair condition. The sheep consumed as much hay as desired, without undue waste. The amounts of grain ranged from 0.35 to 0.60 lb. per head daily, depending on the quality of the hay and the amount of grain necessary to enable the ewes to make normal gains during pregnancy.

Ground oats and upland prairie hay or prairie wool was fed in all trials. The hay varied in quality and composition from year to year and occasionally during a trial, but on the average it was of fair quality, at no time being exceptionally good or poor.

Owing to the scarcity or high price of prairie hay and the abundance of oat hay in Alberta, the latter was included in the rations for the 3rd, 4th and 5th trials. Oat hay (greenfeed) was fed every 3rd day from Jan. 2, 1932, to the end of the 3rd experiment. In the 4th experiment, oat hay was fed every other day throughout the trial. During the 5th experiment, lasting 12 months, prairie, oat, and western rye hays were fed.

The oat hay (greenfeed) was grown on the University farm and cut when the majority of the kernels had reached the dough stage. The leafiness and length of straw were also fairly uniform. The western rye hay was cut following blossoming and was quite well cured, making a satisfactory roughage for sheep.

While no chemical analysis of each hay was made, the average analysis of prairie and oat hay fed at the University of Alberta, and that shown in

"Feeds and Feeding" by Henry and Morrison, Table I, are as follows:—

——	No. of analyses	Moisture, %	Ash, %	Crude protein, %	Fibre, %	NFE,* %	Fat, %
Prairie hay	3 U. of A.	5.4	7.1	6.7	31.5	46.5	2.7
	42 H. and M.	6.5	7.7	8.0	30.5	44.7	2.6
Oat hay	5 U. of A.	10.0	6.2	9.6	33.9	47.4	2.9
	72 H. and M.	12.0	6.8	8.4	28.3	41.7	2.8

* *Nitrogen-free extract.*

Technical casein formed the protein supplement to the diet of Lot VIII.

The "chemically pure" mineral supplements fed to Lot IX varied from year to year, and were as follows:

1st trial: calcium carbonate and sodium sulphate.
2nd trial: calcium carbonate and sodium sulphate.
3rd trial: calcium lactate and sodium sulphate.
4th trial: calcium lactate, mono sodium phosphate and sodium sulphate.
5th trial: calcium lactate, mono sodium phosphate and sodium sulphate.

Length of the Experimental Periods

The length of the experimental periods for Lots I, II, and III were necessarily limited to the late fall and winter months, because both humidity and temperature differences between the comparable groups were directly dependent upon the difference between outdoor and indoor temperatures. During these months there is usually the greatest difference in the average outdoor and indoor (heated pen) temperature. The relative humidity of Lot II (low humidity pen) was dependent upon the number of degrees the outdoor air was raised to equal the desired indoor temperature. Therefore, in order to obtain the greatest differences in both humidity and temperature, the trials had to be limited to those winter months when average outdoor temperatures were low.

While it was not necessary to limit the experimental periods of the nutritional groups to the winter, it was believed that the conditions imposed on them were such as to cause significant effects on wool growth and quality during that period. The growth during the 90- to 100-day period would be one-third to one-quarter the length of each fibre that would be subjected to routine tests. Since, however, it could reasonably be expected that greater differences would develop between the wool of comparable groups by lengthening the experimental periods, this was done in the later trials, the last one continuing for 12 months.

Lots I to III

1st trial: Dec. 3, 1929 to Feb. 28, 1930; 87 days.
2nd trial: Dec. 2, 1930 to Mar. 10, 1931; 98 days.
3rd trial: Nov. 21, 1931 to Mar. 4, 1932; 104 days.
4th trial: Nov. 12, 1932 to Mar. 22, 1933; 130 days.
5th trial: Nov. 8, 1933 to Mar. 24, 1934; 136 days.

Lots IV to IX

1st trial: Dec. 3, 1929 to Mar. 11, 1930; 98 days.
2nd trial: Dec. 2, 1930 to April 24, 1931; 143 days.
3rd trial: Nov. 21, 1931 to April 23, 1932; 154 days.
4th trial: Nov. 12, 1932 to June 2, 1933; 202 days.
5th trial: June 2, 1933 to June 7, 1934; 370 days.

The ewes in Lots V to IX of the 4th trial were left in the same lots throughout the 5th, except for several ewes in the nutrition groups that were re-allotted because of their condition. By continuing these lots into the 5th trial, it was hoped that the results would show an accumulative effect of the experimental conditions imposed.

The authors have failed to find published results of trials in which the effect of controlled temperature and humidity differences were studied. Most nutritional experiments, however, involve periods of six months or longer. Fraser and Nichols (19) compared carbohydrate- and protein-rich diets during a 98-day trial. Darlow *et al.* (11) reported their nutritional trials in which the experimental periods were from 108 to 180 days.

It is recognized that any experiment should be of sufficient duration to affect the performance of the animals in comparable groups. The greater the differences in the conditions imposed, *i.e.*, high *vs.* low protein, the shorter the time that should be necessary to produce an observable effect. It is also recognized that animals may store certain nutritional substances and be capable of performing normally for a longer or shorter period on deficient diets, depending on their reserve, their requirement, or degree of deficiency in one or more nutritional substances.

Method of Sampling Fleeces

To determine accurately the growth and quality of wool produced during the experimental period, samples of wool had to be taken at the beginning and end of each trial. Samples taken at the beginning represented normal wool grown since the previous shearing, during which time all experimental ewes received similar treatment. This first sample was removed from the middle of the right shoulder. At the end of the experiment, a similar sample was removed from the same area on the left shoulder, and represented wool grown prior to and during the experimental period. The difference between the two represented the wool grown during the experiment, and was a measure of the effect of the changed environment.

Experimenters elsewhere have employed different methods of sampling, none of which appeared suitable for the trials now being reported. Most trials covered yearly or half-yearly periods. When this was not the case, much larger numbers of sheep were used.

Fraser and Nichols (19), in their 98-day feeding trial, compared samples of wool that were removed from a tattooed area, prior to and after the trial, and expressed results as "a percentage change upon the conditions at the beginning of the experiment."

The criticisms that could be made of the method of sampling used in these trials are as follows: first, the shoulder sample is not representative of the fleece, and secondly, the wool grown on the right and left shoulders is not identical and therefore cannot be compared.

The mid-shoulder area was chosen for sampling because there was less matting and possibility of wear from rubbing on that part than on any other part of the body. Further, it did not appear necessary to take a sample that would be representative of the whole fleece because it was assumed that if the length, fineness, crimp, yolk, etc., of shoulder wool was affected by the experimental conditions, the wool grown elsewhere would be affected similarly.

At the time of planning the experiment, there was no published proof that wool grown on the right side was dissimilar to that grown on the left, and any cursory study that the authors have made of wool on the animal or of clipped fleeces has not shown obvious variation. Later work, however, has shown that there is a wide variation, even between locks adjacent to each other. According to Fairbanks (16), "when duplicate samples are taken one inch apart and from rather small areas, there are significant differences between the arithmetic mean of their diameters." However, granting the fact, Burns (5) reports that Roberts showed how a reduction from 25% to 5% variation can be secured by "quartering" to secure a composite sample for measurement. Thus, an average mean for fineness was secured that was exceeded by 5% not more than once in 250 times. These references are made to show the extent of the variations and, by the use of improved technique, how arithmetic means with a high degree of accuracy can be obtained.

One could expect, therefore, that by use of the proper technique, comparisons could be made of left and right shoulder samples. With this in mind, special shoulder samples were taken from the left and right sides of five of the experimental ewes and sent to the National Research Laboratories at Ottawa. Diameter measurements of fibres showed average differences as follows: 0.35%, 1.50%, 0.90%, 1.27%, and 0.31%, 120 to 125 fibres taken at random from each sample being measured. For tensile strength, differences of a little over 1% were found. Thus, for these wool characters very little difference existed between the left and right shoulder samples.

For staple and fibre length, differences up to 10% were found when 10 to 12 measurements were made. These might not have been as great had the number of measurements been increased. For grease, suint, dirt, and clean wool, the measurements of which were made in duplicate, the following differences were found between left and right shoulder samples:

—	Av. 5 samples, %	Range, %
Grease	2.27	0.63 to 3.79
Suint	7.04	0.00 to 14.49
Dirt	7.69	3.69 to 16.25
Clean wool	1.98	0.22 to 4.51

It is, therefore, apparent that for most wool measurements there was considerable similarity between left and right shoulder samples.

In experiments conducted for less than a year, a common method of comparison is to remove con-

secutive samples from the same area (6, 7, 18), or the animals are shorn twice yearly (11, 23). This method could have been used had not a number of ewes developed a bare spot in the area from which the first sample was removed. The clipped area was approximately 4 in. by 6 in., and the extremely cold weather which followed sampling may have been the cause of shedding. In some of the black-haired breeds, wool with a large mixture of black fibres grew at the edge of the clipped area. For these reasons it was impossible to take both samples from the same place.

The most satisfactory method of sampling depends upon the type of experiment as well as upon the desired data. It would appear from the literature and facts presented above that the method used was best suited to the trials reported here.

Recording of the Data

All the ewes were weighed for three consecutive days at the beginning and end of the test period and the 3-day averages used as the initial and final weights. Additional weights were taken at the end of each 28-day period in order to follow the gains made by each animal. During the later trials, when the trial extended to and past the lambing period, pre- and post-lambing weights were also taken.

Weights of all feedings were recorded and the amount of hay refused or wasted was noted. Fleece weights were obtained at shearing time.

Breeding records of all ewes were kept, as well as details of their offspring at birth and subsequently.

Any abnormal conditions that developed during the trials, with possible bearing on the results, were noted.

Outdoor temperatures and humidities were observed twice or three times daily. The maximum and minimum temperatures were secured from records of the Field Crops Department. The outdoor humidity readings were taken as described later in this report.

Indoor temperatures and humidities of the heated pens were also recorded twice daily.

As previously mentioned, wool samples from the different groups were forwarded at regular intervals to the National Research Laboratories in Ottawa for critical analysis. For several years, entire fleeces from all the lots were subjected to special grading at the warehouse of the Canadian Co-operative Wool Growers' Association, Weston, Ontario.

The report of the "Laboratory methods used in the measurement of wool characters," being Part II of this series, appears in this issue.

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THE INFLUENCE OF NUTRITIONAL AND CLIMATIC FACTORS ON WOOL GROWTH AND QUALITY

II. LABORATORY METHODS USED IN THE MEASUREMENT OF WOOL CHARACTERS[1]

By P. Larose[2] and A. S. Tweedie[3]

Abstract

The methods described were employed in the laboratories of the National Research Council for the examination of wool samples sent from the University of Alberta. The samples (as mentioned in Part I) were from sheep used to investigate the effect of various environmental factors on wool growth and quality.

The determination of crimp, staple length, fibre length, tensile strength, elongation, fibre diameter, moisture content, wool wax, suint, dirt, and yield of clean wool is described. The methods for the measurement of fibre diameter and for the determination of wool wax, suint, dirt, and clean wool involved new technique and some novel features; they are, therefore, described more fully than the others.

Introduction

Wool clipped from the sheep described in Part I was sent to the National Research Laboratories for testing. There were 72 samples in each of the spring and fall clippings except in the fourth and fifth years, when some of the measurements were carried out on spring samples only.

The large number of samples to be tested made it imperative to use the simplest and most rapid methods consistent with the accuracy desired.

The following characters were measured and reported at one time or other during the experimental period: crimp, staple length, fibre length, tensile strength, elongation, fibre diameter, moisture content, wool wax, suint, dirt, clean wool.

Most of the determinations were carried out by one of the authors (A.S.T.). During the first year, the testing was under the direction of Mr. A. F. Gill, and the authors began work on the problem in the autumn of 1930.

Methods

Conditioning

All physical tests were carried out on samples conditioned at 60% relative humidity and 70° F. except where fibre diameter was determined by the microprojection method, when it was necessary to remove the samples from the conditioning room for measurement. However, in this case, the humidity conditions during the measurement of comparable samples did not vary sufficiently to cause any appreciable change in diameter.

Sampling

Care was taken that the sample for the particular determination to be carried out was representative of the whole sample submitted for test.

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The general procedure was to divide the sample submitted into zones and pick out from each zone a number of locks. A small group of fibres was then taken from each lock and the groups combined. The small sample containing the required number of fibres for any one test was obtained from this composite sample by picking the fibres a few (4–6) at a time. This was the procedure followed in sampling for tensile strength and diameter determinations. For fibre length, the composite sample was omitted and the required number of fibres was picked at random directly from the representative locks. In the case of crimp and staple length determinations, the locks themselves were used. For the wax, suint, and dirt determinations, sampling was simplified by the fact that in the majority of cases the remainder of the original sample, after removal of portions for the other determinations, was utilized.

The number of fibres measured was sufficient in each case to give the desired degree of accuracy.

Crimp

The crimp, as determined in the first year, was obtained by counting the number of waves or kinks in a given length of the individual fibres. The number of readings made on each sample varied between 40 and 60, the observations being made by two people on groups of 20 to 30 fibres. The readings were recorded to the nearest half crimp. The large number of readings was necessitated by the large variation found between individual fibres, *e.g.*, 3–10, 1–7, 2–11, in any one sample. Because of these large variations and because the undulations were generally difficult to distinguish, the crimp in all subsequent samples was determined on the locks instead of on the individual fibres. A rule was held directly against the lock near the root end and the number of undulations in a known length, generally one inch, was counted. Measurements at ten different parts of the sample were usually sufficient to give the required accuracy. In a few cases the crimp was so irregular or ill-defined that the number of waves could not be counted accurately, and no record was made.

Staple Length

The staple length was obtained by holding a rule directly against a lock of wool fibres and noting the distance from the clipped end to the tip. In the case of long wools where the tip of a lock tapered off, a mean length between the longer and shorter parts of the lock was taken as the staple length. The length was read to the nearest millimeter. As in the measurement of crimp, two sets of 20 to 30 observations were made on each sample during the first year. In the subsequent experiments it was found sufficient to carry out 10 measurements on each sample.

Fibre Length

The fibre length was determined by holding the ends of the fibre against a rule by means of forceps, using sufficient tension to remove the kinks without stretching the fibre. A convenient rule for the purpose was made by covering

one face of a wood slat with black paper, along one edge of which was glued a paper scale. The wool fibres were easily seen against the black background next to the scale.

When a Sever fibre length tester was acquired later, the method was checked with this apparatus and no significant difference was found in the results.

The lengths were read to the nearest millimeter, and two sets of 20 to 30 observations were made on each sample in the first year. In the second year, only 10 measurements were carried out on each sample, but in the subsequent experiments this number was increased to 20.

Tensile Strength

The tensile strength of the wool fibres was measured on a Mackenzie type of machine, described by Matthews (4) and by Hill (2).

The distance between the jaws of the machine varied for different samples according to their fibre length, but as far as could be ascertained by a few tests, varying the length within the limits reached in these experiments did not materially affect the results.

In the fourth and fifth years' experiments, instead of measuring the tensile strength on fall and on spring samples, separate measurements were carried out on the root and tip portions of the fibres, the same fibre being used for both determinations. The distance between the jaws was consequently short, being on the average about 2.5 cm. This change was made to decrease as much as possible the error resulting from the variation in strength of fibres and arising from the measurement of strength on two different sets of fibres. It was hoped in this way to render more evident the effect of the factor being investigated. Incidentally, this procedure also lessened the amount of work involved in carrying out the test, since only one sampling and cleaning of the wool was necessary instead of two.

The rate at which the load is applied is an important factor in the use of the Mackenzie machine. Generally, the more rapidly the force is applied to the fibre, the higher is the value obtained. In order to reduce this source of error, all tensile strengths were determined by one operator (A.S.T.) and at a low speed which was kept as constant as possible.

In all cases, the tensile strength was read to the nearest $\frac{1}{2}$ decigram or within the limits 1 part in 100 to 1 part in 500, depending on the strength of the fibre. As a rule, two sets of 25 measurements were carried out for each sample but when the agreement between the two averages was not good, a third and sometimes a fourth set of 25 readings was taken.

Elongation

Elongation was determined at the same time as tensile strength, and therefore, the same remarks regarding number of observations and choice of samples apply here.

The Mackenzie tester has no automatic stop, and the attention of the operator must be continually directed to the elongation scale in order to read the elongation at the moment of rupture. Owing to the low sensitivity of

the scale and the large error possible in reading the elongation, the measurements were discontinued after the first two years.

Fibre Diameter

Three different methods have been used in measuring the diameter of the wool fibres. In the first year's experiments, a number of fibres were placed in a roughly parallel fashion between a microscope slide and a cover glass, the fibres and cover glass being held in position by a little Canada balsam on opposite edges of the cover glass. The diameters were measured by means of a microscope fitted with a micrometer eyepiece. The micrometer was read to the nearest unit and since the magnification was such that one division of the micrometer screwhead corresponded to a cross hair movement of 0.86 μ, the accuracy of the readings was about 2%. Usually two sets of 25 measurements were carried out but when these did not agree within the required limits, additional sets were measured.

For the samples of the second year's experiment and the fall samples of the third year's experiment, the eriometer was used to determine the diameter of the fibres. The instrument was similar to that described by McNicholas and Curtis (5). Cards, having small circular openings of a size corresponding to that of the eriometer, were used to support the fibres. These were placed in parallel sets of ten over each opening.

Although convenient and rapid, the eriometer method was abandoned, for it was found that with fibres which varied widely in diameter in any one sample, consistent readings were difficult to obtain. Apparently, this was because the fibres generally divided themselves into two classes in the formation of diffraction bands. The larger fibres in a set seemed to group themselves in forming one set of diffraction bands while the finer fibres formed another, so that the readings depended on the position of the eye. This is the only fault that has been found with the eriometer and, no doubt, could be eliminated by sorting visually the finer from the coarser fibres of the sample, and then taking separate eriometer readings.

In making measurements with the eriometer, 5 groups of 10 fibres each were tested, 10 readings being taken on each group. These readings were made to the nearest 0.3–0.4μ, or with an accuracy of about 1 part in 100, for the fibres measured.

The remaining samples were measured by a micro-projection method making use of the apparatus described by Larose (3). Fibres from a sample were mounted on cards each holding about 60 fibres. The method of mounting the fibres on the cards is illustrated in Fig. 1, which shows the wooden blocks used when root and tip portions of the fibres were measured separately. This method was followed in the 4th and 5th years' experiments, for the reason given in the discussion of tensile strength. In the earlier experiments, where a single determination was made on each fibre, the central portion was usually measured. This gave rise to an error which will be discussed with the results.

The cards A and B were held by thumb tacks on the blocks C and D, which had two metal strips E and F, notched at $\frac{1}{32}$-in. intervals, to keep the fibres equidistant and parallel. When the fibres were measured at one point only, another block was used, which held apart the two metal strips at a distance equal to that of the width of a card. The two blocks illustrated were held apart at the correct distance, depending on the length of the fibres, by sliding them in or out on two nails G and H. On the outer edge of the blocks alongside the metal strips was placed a sticky wax J made of a

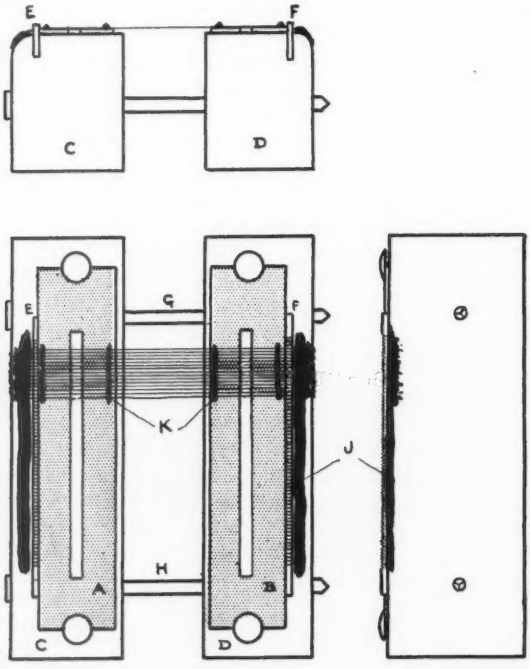

FIG. 1

mixture of beeswax, rosin, and wool wax, or vaseline. The consistency of this wax was such that a fibre could easily be pushed in by the finger, but once embedded it would not easily slip out. The cards were of a length equal to, and of a width about two-thirds that of a microscope slide. They had in the centre a longitudinal slit about 5 mm. wide and slightly longer than the traverse of the mechanical stage on the microscope, so that in moving the stage from one end to the other, the fibres could be brought successively in front of the objective. In mounting, one end of the fibre was fixed in the wax, the fibre passed through opposite notches in the metal strips, and the free end embedded in the wax on the other edge, with sufficient tension to

remove practically all crimp. When the required number of fibres had been mounted in the manner described, they were fixed to the cards on both sides of the slit by means of a hard wax K (rosin + paraffin + beeswax) applied in the molten state. When this wax solidified, the cards were removed and the free portions of the fibres cut away.

The microprojection apparatus gave a magnification of 1000 and the diameter of the image on the screen was measured to the nearest millimeter. This corresponded to an accuracy of 1μ in actual diameter or 1 part in 20 to 40 depending on the size of the fibre. Measurements were carried out usually on 120 fibres, but when the agreement between the two groups of 60 fibres each was not very good, a third and sometimes a fourth group of 60 fibres would be measured.

As some of the results obtained with the eriometer were to be compared with those obtained with the micrometer eyepiece, and others with results obtained with the microprojection apparatus, a comparison was made by carrying out measurements on a few samples by all three methods. The eriometer gave results which on the average were 1.5% higher than those obtained by the microprojection method, and 3.0% higher than those obtained by the micrometer eyepiece. However, a correction of these differences between methods was not essential, since the measurements made were always comparisons and any consistent error would, therefore, cancel out.

Moisture Content

The moisture content of the wool as received was determined in the first two years' experiments only, for it was evident after these determinations that it had no special significance. In the second year the moisture was also determined after conditioning at 60% relative humidity.

The samples, weighing from 25 to 50 gm., were received in sealed tins. On opening the tin, the sample was divided into two portions which were weighed immediately. When the moisture content in the conditioned state was desired, the wool was conditioned at 60% relative humidity for at least a day, or until it reached the equilibrium state. In most cases it was found that more than one day was necessary. After weighing, the samples were dried at 105–110° C. for 3 hr. in a conditioning oven, and again weighed.

The moisture content was calculated on the dry weight to give the regain of the wool, as received and as conditioned at 60% relative humidity.

Wool Wax, Suint, Dirt, and Clean Wool

For the present purpose, wool wax is defined as that part of the raw wool which dissolves in petroleum ether, while suint is represented by the water-soluble portion. The dirt is any matter which does not dissolve in water or in petroleum ether, extraneous to the wool.

The method followed in determining the amount of wool wax, suint, dirt, and clean wool in the raw wool samples was as follows:

Duplicate samples of about 20 gm. of the wool (or the balance, if less than 20 gm. remained after the other determinations) were placed in extraction thimbles (43 × 123 mm.) which had previously been dried in an oven at

105° C. and weighed. The wool and thimbles were dried to constant weight at 105° C., 6 to 7 hr. generally being sufficient. The dry weight was determined without removing the samples from the oven by making use of a balance placed on top of the drying oven and with a suspension passing through the bottom of the balance and the top of the oven. All weights of dried material were obtained in this way so that cooling in a desiccator was not necessary and the change in weight on drying could easily be followed. Designating the weight of the thimble by A and that of the wool and thimble by B, the weight of the dry raw wool is given by B−A. The wool was then extracted with petroleum ether in a Soxhlet for 2 to 3 hr. Owing to the rapid distillation of the solvent and the frequent filling and emptying of the thimble, the extraction was found to be complete in this time, and was equivalent to one of longer duration with a higher boiling solvent such as benzene. The thimble and wool were removed and most of the adhering solvent allowed to evaporate in air, after which drying was carried out in the oven at 105° C., 2 to 3 hr. being sufficient in this case for constant weight to be reached. Designating this dry weight by C, the weight of matter extracted is B−C. The wool was then removed from the thimble and both were washed in three changes of warm distilled water, the washings being collected in a 2-litre beaker and allowed to stand overnight, during which time the dirt settled to the bottom. Washing in warm water removed most of the dirt. The coarse vegetable matter was removed by hand, leaving only a little fine vegetable matter of negligible weight adhering to the wool. The wool and the thimble were then dried at 105° C. to constant weight. Designating the weight of the dried wool by D and that of the thimble by E, the amount of suint plus dirt is given by C−(D+E). The clear liquid in the beaker which had stood overnight was decanted and boiled down to a small volume (10–20 cc.). The residue was filtered on a Buchner funnel fitted to a suction flask and provided with a filter paper slightly larger than the funnel and previously weighed after drying at 105° C. The hot evaporated liquid was also passed through the filter which was then washed with small amounts of hot distilled water and finally dried at 105° C. and weighed. Designating this last weight by H and that of the filter paper by K, (H−K)+(E−A) represents the amount of non-water-soluble dirt, the second term being a correction for any dirt adhering to the thimble. In filtering the dirt from the wash liquors, the best technique found was to use a fairly strong vacuum on the suction flask at the start. Once the suint liquor had begun to filter through, a clamp on the connecting rubber tube was closed. If the filtration slowed up, the clamp was opened again for a short time.

The quantity of suint is determined by subtracting the amount of dirt found from that of suint and dirt previously determined. The amount of clean wool is, of course, given directly by D.

This method differs from that of Sutton (6) and the later modification described by Hill (1) in that petroleum ether is used as the wax solvent instead of ether as in the first, or benzene as in the second method, while the time of extraction is much shorter. The water treatment is also different,

because extraction with water has been found to have the following disadvantages. The thimbles are generally useless after one extraction. The method is long, and the apparatus has to be specially insulated to obtain the necessary boiling. The prolonged boiling-water treatment renders the wool unsuitable for further tests. It has also been found difficult to prevent dirt from siphoning over with the water. In a comparison of the two methods, it has been found that the simple washing with warm water gives results which are quite satisfactory. The determination of the wax and the suint by difference seems also to be more advantageous than the method of evaporating the respective solutions to dryness, as employed by Sutton and described by Hill. It has not been deemed necessary to carry out the alcohol extraction of any water-soluble matter removed with the wax, as this was found to be very small. The nature of this material seemed also to be different from that of the water-soluble suint, so it is questionable whether it should be classed as such.

The following figures show the results obtained with two wools in the comparison of the method described above (designated N.R.C. in the table) with that described by Hill. The figures are means of duplicate determinations and are calculated on the original weight of the dry raw wool.

Sample	698		699	
Method	N.R.C.	Hill	N.R.C.	Hill
Wax (by diff.)	13.5%	(14.0)%	10.5%	(10.9)%
Wax (by evap'n.—alc. ext.)		12.8		10.1
Suint (by diff.)	18.1	(18.0)	11.6	(12.1)
Suint (evap'n.+alc. ext.)		18.1		11.6
Dirt	4.0		5.0	
(by diff.)		5.0		6.0
(by diff. using bracketed fig.)		(3.9)		(4.7)
Clean wool	64.3	64.1	72.8	72.3

The figures given in brackets are the results of determinations not included in Hill's method. The slight deviation of the totals from 100% is due to omission of the second decimal.

The smaller amounts of wax obtained by the evaporation method are probably due to decomposition accompanying drying. For this reason, the determination of these constituents by difference was considered preferable. For the same reason, the amount of dirt determined by difference is likely to be too high.

Part III of this series will deal with the results of these experiments and a general discussion of their significance.

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